Phytochemical and Molecular characterization in selected species of Ocimum in and around of Munger District

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August 2025

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(M. A. Jahanger)

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PREFACE

Development in the area of biotechnology, requires a sound understanding of Principles underlying the biochemical, phytochemical and molecular basis of life processes, Investigational approach based on fundamental scientific principles from the basis of experimental biotechnology.

In the long struggle to achieve mastery over powerful forces of nature, man have always terms to plant for help. This is especially so, when he was struck with ailments, both physical and mental. Nearly all cultures, both ancient and recent, have used plants as source of medicine. We would never be able to say how exactly the ancient discovered the medicinal properties of herbs. Probably it started with ancient beliefs, myths and lords, got involved with astrology and others occult practice, developed into folk medicine and herbalism, and finally gave rise to traditional systems of herbal medicine.

In the present research work I study on Ocimum plant as a herb. This thesis deals with phytochemical and molecular characterization of four selected species of Ocimum, in which primary and secondary metabolites, plant pigment, oil extraction, DNA isolation, Gel Electrophoresis, PCR and Sanger sequencing have been analyzed with perfect techniques and in simple language with flow sheet of practical work.

I hope that the students, researchers and teachers will enjoy reading this thesis, as an important tool in practicing practical work.

Readers are most welcome to appraise me of their critical appreciation of this research work.

- M. A. Jahanger

List of abbreviation

CTAB	:	Cetyl Trimethyl Ammonium Bromide
DNA	:	Deoxy ribose Nucleic Acid
dNTP	:	Deoxy Nucleotide Tri phosphate
ddNTP	:	Double Deoxy Nucleotide Tri phosphate
DW	:	Distilled water
Et Br	:	Ethidium promide
EDTA	:	Ethylene diamine tetra acetic
FC	:	Folin ciocalteau
FW	:	Fresh Weight
Fig	:	Figure
KVK	:	Krishi Vigyan Kendra
L. or Linn.	:	Linnaeus (Carolus Linnaeus)
OD	:	Optical Density
O.b	:	*Ocimum basilicum* L.
O.g	:	*Ocimum gratissimum* L.
O.t	:	*Ocimum tenuiflorum* L.
Rbcl	:	Ribulose biphosphate carboxylase large chain
RFU	:	Relative Fluorescence Unit
% T	:	Percent Transmission
T	:	Transmission
TAC	:	Total Alkaloid Content
TCA	:	Tri carboxylic acid
T. T	:	Test Tube
TLC	:	Thin Layer Chromatography
TAE	:	Tris Acetic acid EDTA
TBE	:	Tris Boric acid EDTA
Taq	:	Thermus aquaticus
TEA	:	Toluene + Ethyl acetate + Acetic Acid

ABSTRACT

The district of Munger is located in the Eastern part of Bihar, India. It is hemmed among the Ganges in the North, Bhagalpur & Banka in the East, Lakhisarai in the West and the District of Jamui in the south. It is located between $24^0$30' and 25^0 North Latitude and between $86^0$30' and 87^0 East Longitude.

The present doctoral investigation aimed to perform a detailed quantitative analysis of key biochemical constituents—namely proteins, sugars, alkaloids, flavonoids, glycosides, phenols, steroids, plant pigments, and essential oil components—in three important Ocimum species (*O. tenuiflorum* L., *O. basilicum* L., and *O. gratissimum* L.), The findings offer substantial insight into species-specific metabolic profiles and their responses to environmental influences, laying a strong foundation for targeted utilization in nutraceutical, pharmaceutical, and agronomic applications.

Protein concentration was estimated using the Lowry method, a sensitive and validated approach leveraging the reaction between peptide bonds, copper ions, and Folin–Ciocalteau reagent. The assay showed high linearity and accuracy, confirmed through a standard curve developed using Bovine Serum Albumin (BSA) within a range of 10–100 µg. A steady increase in optical density (0.031 to 0.292) correlated with a decrease in transmittance (%T from 93% to 51%), reinforcing the method's reliability. *O.basilicum* L. showed high sugar level (2.88 mg/100 ml), while *O.gratissimum* L. records a slightly lower sugar concentration of 2.76 mg/100mg.In contrast, *O.tenuiflorum* L. exhibited the lowest sugar content with 2.58 mg/100mg.Flavonoid content was measured using the Aluminum Chloride ($AlCl_3$) colorimetric method with quercetin as the standard reference. Optical density values from the assay revealed a clear gradient of flavonoid concentration among the species.

O. tenuiflorum L. (Shyama Tulsi) exhibited the highest flavonoid content (32 µg/ml, OD 0.34), indicating robust secondary metabolism and suggesting strong antioxidant, anti-inflammatory, and pharmacological potential. This was followed by *O. basilicum* L. (26 µg/ml), and *O. gratissimum* L. (24 µg/ml). These findings underscore the medicinal value of *O. tenuiflorum* L. as a leading source of bioactive compounds and validate the spectrophotometric method as a dependable tool for phytochemical quantification. Paper chromatography confirmed the presence of four key pigments—Carotene, Xanthophyll, Chlorophyll-a, and Chlorophyll-b—across all species, each displaying distinct R_f values and mobility patterns. Quantitative data revealed that *O. gratissimum* L. had the highest total chlorophyll content across all seasons and locations, indicative of superior photosynthetic performance. *O. basilicum* L. followed closely, while *O. tenuiflorum* L. recorded comparatively lower chlorophyll levels.

The genus Ocimum comprises of a large number of species which are widely cultivated in different parts of the world for its pharmaceutical, nutraceutical and other commercial importance. A number of biological activities such as antimicrobial, cytotoxic, antinociceptive, anti-inflammatory, hypoglycemic, hepatoprotective and antioxidant have been ascribed to Ocimum which can be linked to the occurrence of biologically active phytochemicals like phenolic acids, essential oil, flavonoids, and terpenes hence making it a potential source for discovery and development of novel drugs. The present review covers the successive advances in the traditional medicinal aspects, photochemistry, bioactivities and their bioavailability of three Ocimum species *Ocimum basilicum* L., *Ocimum gratissimum* L. *and Ocimum tenuiflorum* L., in order to explore their applications in versatile industries.

Present work is based on phytochemical and molecular characterization in selected species of Ocimum in and around of Munger District. In this regard three species have been selected namely *O. tenuiflorum* L., *O. basilicum* L. *and O. gratissimum* L.. In phytochemical characterization, primary and secondary metabolites have been analyzed and it was found that out of three selected species,

O. tenuiflorum L. has lowest amount of sugar, *O. tenuiflorum* L. has highest amount of flavonoids and terpenoids whenever in Molecular characterization, DNA has been isolated and separated in all the three selected species of Ocimum, after agarose electrophoresis it has quantified and found that *O. tenuiflorum* L. *and O. gratissimum* L. have same value, i.e. 166ng/ µl DNA after valuation of O.D 332, and in *O. basilicum* L. has value,i.e. 165ng/ µl DNA after valuation of OD 330. Sanger sequencing has also been done to know the sequencing of nitrogenous base, where gene sequencing was observed as electropherogram. In this electropherogram all the four nitrogenous bases indicated in different color. Red peak stand for thymine (T), green peak stand for adenine (A), blue peak for cytosine (C) and brown peak stand for guanine (G). The height of the peak reflects the signal strength the signal strength shows on x-axis from 100 to 1000 RFU. The position of the peak on the x-axis indicates the location of the nucleotide in the DNA sequence. The sequence characterizes by having multiple peaks. It was observed that out of 320 nucleotides, there are A=92, T=91, G=63 and C=40 in *O. tenuiflorum* L. In *O.basilicum* L., the nucleotide composition was A=90, T=90, G=72 and C=68 while in *O.gratissimum* L. the values were A=81, T=88, G=75 and C=76 peaks. Highest length of peak up to 320 nitrogenous bases, with a fluorescence intensity touching 800 RFU. Among these, guanine (G) showed the most significant representation with 6 peaks, followed by thymine (T) with 1 peak, whereas cytosine (C) and adenine (A) were not represented at this intensity. *In Ocimum gratissimum* L., 47 peaks were observed in which guanine (G) with 22 peaks, adenine (A) with 13 peaks, cytosine (C) with 8 peaks, and thymine (T) with 4 peaks.

Notably, *Ocimum tenuiflorum* L. (Shyama Tulsi) exhibited the highest number of nitrogenous bases reaching an RFU (Relative Fluorescence Unit) threshold of 800, with a total of 79 peaks: thymine (T) = 25, guanine (G) = 16, cytosine (C) = 19, and adenine (A) = 17.

These Sanger sequencing results indicate that *O. tenuiflorum* L. possesses the highest number of nitrogenous bases with the longest and most intense peaks,

surpassing the 800 RFU threshold, suggesting a stronger signal and possibly higher-quality or more abundant DNA fragments in this species. Electropherogram confirms the successful amplification and sequencing of the *rbcl* gene in *Ocimum gratissimum* L., with acceptable sequence integrity for bioinformatics analyses despite some minor signal degradation in the later regions. The data contributes significantly to the molecular characterization and species-level identification of *O. gratissimum* L. within the broader framework of Ocimum biodiversity studies.

This study provides a significant step toward evidence-based utilization of Ocimum species in traditional and modern herbal systems, supporting biodiversity conservation, economic botany, and sustainable agriculture.

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CHAPTER-1

INTRODUCTION

I N T R O D U C T I O N

The plant kingdom is an excellent source of potential drugs (*Borah and Biswas-2018*). Over the past 20 years there has been increased interest in the investigation of Natural materials, as sources of antibacterial agents (*Okema et al 2003*). In the recent year the development of resistance of pathogens against antibiotic has become a difficult issue caused by the indiscriminate use of modern antibiotic (*Arya & yadav 2010, M Rashid & Islam 2012*), Considering the high cost of the synthetic drugs and other side effect, wide varieties of natural plant, Tulsi is one of them which can be considered as a vital source for antimicrobial agents (*Geyied et al 2005*). Tulsi is an important symbol of Hindu Religious tradition, its other name is Vishnupriya, means the one that pleases Lord Vishnu,found in most of the Indian homes and worshipped (*Govind Pandey & Madhuri 2010*). It is also referred to as "Reihaan" in the Holy Quraan, specifically in Surah Rahman, where it is considered as sacred plant, also known as "paak paudha"in Urdu.

Ocimum species commonly known as Tulsi, is an herbaceous perennial belonging to the family Lamiaceae and is considered as one of the most important sources of herbal medicine (*Borah and Biswas 2018*). Tulsi is used in different forms like aqueous extracts from the leaves (fresh or dried as powder).These extracts are used in herbal teas and also mixed with other herbs as well as honey to enhance the medicinal value. Traditional uses of Tulsi aqueous extract include the treatment of different types of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart disease (*Pattanayak et al 2010*). Oils extracted from the leaves and inflorescence of Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents (*Singh et al 2010*).

However, due to over population and continuous exploitation of these herbal reserves along with their repeated traditional knowledge are depleting day by day.

Keeping in view, the biodiversity of India and to further its potential in ethno pharmacological area, Present study is an attempt to study on the basis of phytochemical and molecular characterization for the three following important species of Ocimum found in Munger District.

1. *Ocimum tenuiflorum* L.
2. *Ocimum basilicum* L.
3. *Ocimum gratissmum.* L.

Medicinal plants have been in use by mankind since time immortal to prevent and manage diseases and with the progress of modern pharmaceutical.The bioactive compounds derived from these plants are being used as major components in the contemporary medicines. Among these, primary and secondary metabolites obtained from the genus 'Ocimum' holds great value for agricultural as well as commercially important industries like pharmaceuticals, culinary and cosmetics due to their high performing metabolites.

Ocimum L. is a genus of annual, aromatic, and perennial shrubs and herbs in the Lamiaceae family whichis regarded as the 'King of Herbs, due to the exceptional properties, which makes it suited for its extensive usage in traditional medicine, perfumery, cosmetic, food and pharmaceutical industries (*Simpson, B. B. et al. 2001*).Ocimum, is also known as basil, is one of the most genetically diverse genera, with over 150 species reported so far (*Makri, O. et al. 2008*). The term basilikos means "royal" in Greek (*Putievsky, E. et al.1999*) are found in Africa, Asia, Central and South America. There are nine types of Ocimum species in India, among which

O.minimum L., *O.americanum* L. & *O.africanum* L. are rare in nature (*Balyan, S.S. et al. 1988*). Our study examines the phytochemical diversity of three commonly cultivated and extensively used Ocimum species which are *O. tenuiflorum* L., *O. basilicum* L., *and O. gratissimum* L..

Ocimum tenuiflorum* L.*: It is commonly known as Shyama Tulsi. It is a short-lived perennial plant with over 150 varieties. Tropical and subtropical areas of Africa, Sri Lanka, SW Asia, Himalaya, India, China, Burma Thailand Malaysia Philippines and Indonesia are home to this plant (*Ashwini, A. et al. 2021*). It is commonly available in Cuba (*Oviedo, P. et al. 2012*) and Puerto Rico, India, Malaysia, and Guam (*Randall, R. A. et. al 2012*), growing in agricultural regions and home gardens, it is widely used for religious purposes too, apart from being used by perfumery industries.

***Ocimum basilicum* L.**: It is commonly known as Sweet basil. It is an annual spicy plant, native to the Indian subcontinent. It is a tropical herb distributed throughout Southeast Asia, Europe and Central Africa. The warm and temperate nations that grow it commercially include Morocco, Egypt, and Indonesia. It is also cultivated in New Mexico, Arizona, North Carolina, and California, where the leaf is used for various medicinal purposes (*Prakash, V. et al. 1990*).

Ocimum gratissimum* L.*: It is commonly known as African basil. It is an aromatic medicinal plant, majorly popular in Kenya as well as other places in Sub-Saharan Africa (*Matasyoh, L.G. et al. 2008*). It is grown for medicinal and culinary applications in various parts of the world (*Iwu, M.M 1993*). It belongs to South- East Asia and India and has grown and naturalized in South America, China, The Caribbean, New Zealand, Australia, and several Indian and Pacific islands. It escapes cultivation and now grows as a weed in disturbed regions, waste areas, pastures, and along roadsides, as well as infiltrating disturbed natural vegetation, savannas, coastal thickets, and

riparian areas. Seeds are small and plentiful in this species, and they are easily disseminated by gravity, animals, human activities, and as a contaminant in soil and garden detritus. Once established, it can develop dense monospecific culms that outcompete native species and diminish biodiversity in the area (*Rojas – Sandoval, J. 2021*).

TRADITIONAL USES OF OCIMUM SPECIES

Plants are known to be powerful biochemists and thus have been used in phytomedicine since the dawn of time. A diverse range of commercially important phytochemicals are being extracted from plants. Ancient scholastic works such as the Atharvaveda, Ayurveda and many more included a wealth of knowledge on preventive and therapeutic remedies from plant sources. Traditional remedies derived from these sources are still used by a large section of the population throughout the world. According to WHO estimates, 80 per cent of the population of the world relies on the herbal traditional system of medicines for basic healthcare, therefore research into the qualities and uses of these natural medicinal plant resources is gaining attention (*Aggarwal, A. et al. 2015*). Around 13000 plant species have been used as medications around the world and are being harvested for their different parts (*Cragg, G.M. et al. 2001*). To highlight the importance of traditional medicine, national governments have created a traditional medicine regulatory council under the supervision of their health ministries, in order to capitalize on the many advantages of medicinal plants. This may be due to the fact that traditional medicine has been used for a long time, even before mainstream medicine was established (*Okafor, J.I et al 2001*). Chronic lifestyle illnesses are the leading cause of morbidity and death worldwide, and many of them are treatable with good lifestyle choices and use of adaptogenic like *Ocimum* or Basil. Basil is widely used as traditional medicine and also being consumed as seasoning. When the seeds are soaked in water, they turn mucilaginous and edible, and the leaves are eaten fresh or

cooked. It is used to treat digestive ailments such as stomach pain and diarrhea, renal illnesses, and infections in Ayurveda and traditional Chinese medicine. It is used to cure whooping cough and a range of fevers in traditional medicine systems. Leaf decoction is used to cure cold and coughs (*Dzoyem, J.P et al 2017*). Sushruta, an ancient Ayurvedic surgeon, categorised *Ocimum basilicum* as a green leafy vegetable, whereas Bhavamisra, a well-known Ayurvedic Acharya, called *Ocimum basilicum* 'barbari.' To name a few, it is mentioned in Sushruta Samhita, Charaka Samhita, Bhavaprakasham, and Ashtangahridayam, Ayurvedic literature (*Pushpagandan, P. et al 1993*). In certain Mediterranean regions, such as Eastern Morocco, they are used to reduce plasma lipid levels (*Zhang, J.W et al 2009*). The whole plant is also used in Ayurvedic medicine to treat bronchitis, asthma, giddiness, ophthalmia, malaria, catarrh, otalgia, dyspepsia, spasmodic and cephalalgia abnormalities (*Udayan, P.S et al 2009*). Many Tribal communities in India use sweet basil to cure headaches, inflammation, earaches, coughs, colds, fevers, snake bites, and rabies. Its leaves have also been used to treat diarrhoea, dysentery, constipation, gas, and worms, as well as acts as an analgesic and insect repellent. They are also used to cure bronchitis, colds, flu, coughs, sinusitis, rheumatism, muscular pains, gout, and general fatigue. According to some accounts, it may also be used to cure warts, insect bites, and skin blemishes (*Pushpagandan, P. et al 1993 & Simon, J.E et al 1999*). A combination of leaf extract and camphor may be used to alleviate nasal haemorrhage. It is said to brighten the eyes and to be a good cure for ringworms, scorpion stings, and snake bites. Seeds from the plant acts as a demulcent in genito-urinary illnessand also given to relieve childbirth after pains, and in fever. The herb has been described as a stimulant, carminative, diaphoretic, diuretic expectorant, and antipyretic (*Kirtikar, K.R & Basu 1935*).

O. tenuiflorum L. has been utilised in Ayurveda for thousands of years, due to its numerous medicinal properties. Tulsi, or holy basil, is valued in the East for its religious and spiritual purity, as well as its role in Ayurvedic and Unani systems of holistic health and herbal treatment. Tulsi is an adaptogen, which means that it helps in reducing stress by regulating a number of physiological processes. It is considered as ‘elixir of life'. Tulsi is used to treat colds, headaches, stomach problems, inflammation, heart disease, poisoning, and malaria. *O. sanctum* is typically consumed in the form of herbal tea, dried powder, and fresh leaf. It leaves have been combined with stored grains for millennia to keep insects at bay (*Pattanayak, P. et al 2010 & Biswas, N.P et al 2005*).

O. gratissimum L. has a long history of use as a condiment and for treating ailments such as pyorrhea, bronchitis, and dysentery in Africa (*Ogunniran, K.O. 2009*).The dried leaves are used to treat headaches and fever (*Iwu, M.M. 1993*). Inmany communities around the world like the Igala community in Kogi State, leaves and roots are used to cure diabetes, gastrointestinal issues, gonorrhoea and malaria (*Aniama, S.O. et al 2016*),.In parts of Africa, leaves are used for treating of sexually transmitted infections (*Gbadamosi, I.T., & Egunyomi 2014*), and as significant antifungal. Traditional medicine practitioners employ leaf extract in the treatment of skin illnesses such as Eczema (*Mbakwem-Aniebo, C. et al 2012*). It is used as a febrifuge and also in the treatment of respiratory disorders, in parts of central Africa (*Martins, A.P. et al 1999*). In Brazil, it is used in treating bronchitis, coughs, sore throat, and also in flavoring soup and meat (*Okoli, C.O. 2010*).

The whole plant is used to treat sunstroke, headaches, stomach-ache and influenza in South Asia, while the seeds are used to treat gonorrhoea (*Simbo, D.J. 2010*). Traditional practitioners in northwest Africa utilize the plant in treatment of dystocia, vomiting, dysmenorrhea, mycoses, digestive

problems, hemorrhoids, dysentery, abscess, and typhoid fever and also as an insect repellent (*Simbo, D.J. 2010*). In India, this plant leaves have also broad folklore medicinal implications and is used in treating diarrhea, fever, headache, pneumonia (*Prabhu, K.S. et al 2009*). Also used as an antidote in snake bites in some regions. Seed extract with sugar is used as a nerve tonic during hot summer days, in central India and an extract of the leaf is applied in cuts to stop bleedings (*Malaiya, P.S. 2016*).

In northern parts of India, the plant is grown commercially and widely used for aromatherapy purposes, perfume for herbal toiletries and essential oil. Whereas in Central India, the local people of the Bundelkhand region applies leaf decoction on the skin of cattle to treat ectoparasite (*Verma, R.K. 2014*). The Manipuri community of Barack valley, in Assam, uses leaves with honey to cure fever. In Tripura, they also use zinger with Tulsi leaves and honey to cure fever, diarrhea, headache, eye problems, and upper respiratory tract illness and skin problems.

The Yandi Tribe resides in the Eastern Ghats of Andhra Pradesh uses the juice of the whole plant to treat cough and cold. While the Nan cowry community of Nicobar Islands utilizes the plant orally for breathing disorders and leaves are also used in treating rheumatism in some parts of Odisha (*Panda, S.P. et al 2014*)

O. tenuiflorum L. is best recognized for its adaptogenic stress-relieving qualities to normalize physiological functions and restore balance. The fresh leaf is used in the treatment of common colds, fevers, inflammation, and diabetes, just like other *Ocimum* spp., and root extract is utilized as a sexual stimulant in various parts of West Bengal, India (*Chowdhury, T. et al 2017*). Methyl eugenol is a high-value aroma chemical discovered in *O. tenuiflorum* which is utilised as a flavouring ingredient in jellies, baked products, chewing gum, non-alcoholic drinks, sweets, pudding, relish, and ice cream (*Machado, M.I.L. et al 1999*).Because of its incredible medicinal potential,

holy basil has the potential to be a high-value cash crop that can be produced in the United States utilising quality and traceability standards. According to the American Botanical Council, tea sales in the United States surpassed $15 billion in 2013, and tea consumption is expected to rise in the coming years (*Keating, B. et al 2013*).

Geography of Munger

The District of Munger is located in the Eastern part of Bihar, India. It is hemmed among the Ganges in the North, Bhagalpur & Banka in the East, Lakhisarai in the West and the District of Jamui in the South. It is located between $24^0$30' and 25^0 North Latitude and between $86^0$30' and 87^0 East Longitude

Climatologically, the District of Munger comes under subtropical monsoon climate. January is the coldest months with mean daily minimum and maximum temp of 8.5^0c and $24^0.4^0$c May and June are the hottest months with mean daily minimum and maximum varying 28.8^0c and 51^0c.

Figure 1.1: Map of Bihar

Figure 1.2: Map of Munger

1.1 OVERVIEW

The Genus Ocimum comprises many species, widely cultivated in different parts of the world for their pharmaceutical, nutraceuticaland other commercial important, many biological activities such as antimicrobial, cytotoxic, anti-inflammatory, hypoglycemic, hepatoprotective and antioxidant have been ascribed to Ocimum.

Tulsi is an important symbol of Hindu Religious tradition, its other name, Vishnupriya, means this one that pleases Lord Vishnu found in most of the Indian homes and worshiped. It is also known as "Paak Paudha" or "Jannat ka Paudha" in religions book of Quraan in Surah Rahman as the name "Reihaan"

Classification:

Division	Magnoliphyta
Order	Lamiales
Class	Magnoliopsida
Family	Lamiacea
Genus	*Ocimum*
Species	*tenuiflorum* L./*gratissimum* L /*basilicum* L etc.

Synonyms of Tulsi (Ocimum)

English	Holy Basil
Hindi	Tulsi
Sanskrit	Tulasi
Urdu	Reihaan
Arabic	Reihaan
Punjabi	Tulsi

Ocimum commonly known as "Tulsi"

Species of Ocimum (Tulsi): - The genus Ocimum commonly known as basil, encompasses a variety of species and cultivarse with diverse characteristics and uses.Following are the important species of Ocimum

1. *O. tenuiflorum* L.
2. *O. basilicum* L.
3. *O. citriodorum* L.
4. *O. americanum* L.
5. *O. gratissimum* L.
6. *O. cinnamon* L.
7. *O. kilimandscharicum* L.
8. *O. cannum* L.
9. *O. labiatum* L.
10. *O. selloi* L.
11. *O. minimum* L.

Out of the above species *O. tenuiflorum* L., *O. basilicum* L. *and O. gratissimum* L. are found in and around of Munger District, commonly known as Shyama Tulsi, Sweet Tulsi and Van Tulsi respectively

Organization of Thesis

This thesis is organized into seven chapters.Chapter 1 provides the introduction, Rationale of study & objectives, Chapter 2 reviews relevant

literature on the phytochemistry, oil extraction, Chromatography technique & molecular characterization, Sanger sequencing & uses of Tulsi. Chapter 3 details the materials and methods used in the research. Chapter 4 presents the results and discussion. Chapter 5 Conclusion, Chapter 6 Future prospects & Chapter 7 includes refrences.

- Moreover, ethanomarphological importance of Ocimum species described in following table.

Table 1.1: Ethnopharmacological importance of various Ocimum spp. &Source

Source	Biological activity	Ethnopharmacological Importance	References
O. gratissimum L.	Anti-inflammatory and anti-nociceptive	Extract use locally to treat painful and inflammatory disorders	Thanko, Y. et al 2008
O. gratissimum, L.	Antioxidant	Free radical scavenger, reducing the consequences of free radical in the body	Njoku et al 2011
O. gratissimum L.	Antiurolithiatic	Inhibition of calcium oxalate crystal formation	Agarwal, K. et al 2014
O. gratissimum L.	Antifungals,	Topical application of leaves, stem and flowers to the skin for several days can help to eradicate foul body odour	Harborne, J.B et al 1993
O. teuiflorum L.	Anti-inflammatory, antioxidant and immune-modulatory and antistress	Taken in many from, as herbal tea, dried powder of fresh leaf.	Sen, P. et al 1992
O. gratissimum L.	Radioprotective and anti-carcinogenic		Devi, P.U et al 2001
O. gratissimum L.	Antiasthmatic, antikaphic, bronchitis, rheumatism and pyrexia	Indian Materia Medica describe the use of aqueous, hydroalcoholic and methanolic extract of leaves	Siva, M. et al 2016

O. gratissimum L.	Antidote	For dog bite, scorpion bite, snake bite and insect bites,	Godhwani, S. et al 1988
O.basilicum L.	As a tonic and vermifuge, good for treating dysentery, nausea and flatulence.	Decoction and infusion are the most common method of preparation as a fresh or dry leaf. The plant is effective in treatment of stomach problems	Nadkarni, K. M. 2010
O.basilicum L.	Antioxidant	Methanolic and queous extracts of leaves may be attributed to their phenolic content as well as the presence of the flavonoids, tannins and essential oils	Benabdullah, H. et al 2020
O.basilicum L.	Anti-inflammatory, antioxidant	Hexane and ethanol fruit extracts	Osei Akoto, C. et al 2020
O.basilicum L.	Anthelmintic, diaphoretic, antiemetic, antidiabetic, and antidiarrhoeaic	In Annam infusion of the whole plant	Bilal, A. et al 2012
O. tenuiflorum L.	Antitussives	Highest fidelity level (FL) of 100% in Bandarban Hill Tracts (BHT), Bangladesh	Kadir, M. F et al 2014
O. tenuiflorum L.	Analgesic and anti nociceptive	Management and treatment of Pain in the topical of Mauritius	Sreekisoon, D.P et al 2014

1.2 RATIONAL OF THE STUDY

Research on the Tulsi plant is justified because of its many potential health benefits and wide range of therapeutic application.

i. It is easily available.
ii. Tulsi can help with psychological stress, including anxiety and depression.
iii. Anti-inflammatory -Tulsi can help relieve inflammation and protect against gastric ulcers
iv. Antimicrobial – Tulsi can help fight a range of human and animal pathogens.

v. Anticancer – Tulsi may help prevent the development of lung, breast, liver and oral cancer.

vi. Antioxidant – Tulsi contains antioxidants that can help protect against chemical and physical stress.

vii. Tulsi can be used as a hand sanitizer mouthwash and water purifier.

Therefore, we can say that Tulsi is an effective treatment for life style related chronic diseases including diabetes, metabolic syndrome and psychological stress.

Oil extracted from the leaves and inflorescence of tulsi have been claimed to have numerous, useful properties, including as expectorants, analgesics, anti-emetics, and hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents (*Singh et al 2010*).

However, due to over Population, organization and continuous exploitation of these herbal reserves, the natural resources along with their repeated traditional knowledge are depleting day by day.

Keeping in view the biodiversity of India and to further its potential in ethnopharmacological area, Present study is an attempt to study three medicinally important species of Ocimum of Munger, based on Phytochemical and Molecular characterization. These species are

1. *Ocimum tenuiflorum* L.
2. *Ocimum basilicum* L.
3. *Ocimum gratissimum.* L.

1.3 OBJECTIVE

The present doctoral research successfully fulfilled the following five key objectives, focusing on the phytochemical and molecular characterization of three Ocimum species *O. tenuiflorum* L., *O. basilicum* L., and *O. gratissimum* L., collected from three representative locations in Munger District, Bihar.The main objective of the research is to discover new facts or to make

fresh interpretation of known facts, for this the whole work plan of this research is divided into following five points.

i. Collection and identification of selected plant.
ii. Phytochemical screening in selected plant species.
iii. Analysis of plant Pigments.
iv. Ethnopharmacological study through oil extraction by Clevenger apparatus.
v. Molecular characterization of selected plant species.

CHAPTER-2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Review of Literature and Research Gap

Several studies have highlighted the medicinal importance of Ocimum species andtheir rich phytochemical profiles. Research has shown that compounds like Eugenol, Ursolic acid, Linalool, and various Flavonoids are responsible for the antimicrobial and anti-inflammatory properties of Tulsi. However,most of thesestudies have either been limited to one or two species or focused on single-season collection, often overlooking the influence of seasonal and geographical variability.

Moreover, molecular studies in Ocimum have primarily been aimed at taxonomy and chemotype identification rather than understanding ecological adaptation.The Munger District, despite its rich flora and varied ecosystems, remains largely under explored in this context.

This study addresses these research gaps by:

- Including multiple Ocimum species.
- Selecting ecologically distinct locations for sample collection.
- Conducting seasonal collections to examine temporal effects.
- Employing both phytochemical and molecular tools for a comprehensive analysis.

2.1 Introduction of Ocimum species

The genus Ocimum comprises of a large number of species which are widely cultivated in different parts of the world for its pharmaceutical, nutraceutical and other commercial importance. A number of biological activities such as antimicrobial, cytotoxic, anti-nociceptive, anti-inflammatory, hypoglycemic, hepatoprotective and antioxidant have been ascribed to Ocimum which can be linked to the occurrence of biologically active phytochemicals like phenolic acids, essential oil, flavonoids, and terrenes hence making it a potential source for discovery and development of novel drugs. The present

review covers the successive advances in the traditional medicinal aspects, photochemistry, bioactivities and their bioavailability of four Ocimum species are *Ocimum basilicum, Ocimum sanctum, Ocimum gratissimum and Ocimum tenuiflorum* in order to explore their applications in versatile industries. Furthermore, this thorough compilation will be used as a potential resource for further Ocimum related medication development and research and also to identify the research gaps to fill.

A.N.M. Mamun-or-Rashid et al (2013), reported that *Ocimum sanctum* (also known as *Ocimum tenuiflorum,* Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. The sacred, Tulsi is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine. In traditional system of medicine, different parts of *O. sanctum* have been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The *O. sanctum* has also antifertility, anticancer, antidiabetic, antifungal, antimicrobial, analgesic and antispasmodic actions. Locally, it is taken in forms, as herbal tea, dried power or fresh leaf.

Agarwal, K, et al (2014), reported that *O. gratissimum* has been used to cure a variety of ailments, including urinary stone disorders. The nucleation test and synthetic urine assay were used to evaluate the prevention of calcium-oxalate crystal formation by *O. gratissimum*. The goal of the nucleation assay was to assess the effect of different concentrations of the extract (100-1000 mg/ml) on *in vitro* crystallization of calcium oxalate, whereas the percentage inhibition and formation of calcium oxalate monohydrate crystals from synthetic urine were tested at varied percent concentrations of extract (25-100 percent). In both the assays, the percent inhibition of calcium oxalate crystal formation was found to be directly proportional to the plant extract concentration, with a maximum inhibition of 66.08 % at 1000 mg/ml in the plant extract assay and 62.07 % at 100 percent in the synthetic urine assay.

As a result, *O. gratissimum* was discovered to be a strong and effective antiurolithiatic agent, consistent with its traditional use

Aniama et al. (2016) studied that, in many communities around the world like the Iqala communities in Kogi state, leaves and root of Tulsi are used to cure diabetes, gastrointestinal issues, gonorrhea and malaria.

Balyan et al. (1988) found that there are nine Ocimum species in India, among which *O.minimum, O. americanam* and *O. africanum* are rate in nature whenever *O.tenuiflorum, O.basilicum, O.santum* and *O.gratissimum* are commonly cultivated and extensively used.

Biswas et al. (2005) justified that tulsi used to treat colds, headaches, stomach problems, inflammation, heart disease, poisoning and malaria.

Doss, A & Borah, R (2009) revealed that the plant kingdom is an excellent source of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Medicinal plants are rich source of different types of medicines and produce various bioactive molecules. Herbal plant extracts are very useful and are the major sources of medicine which play vital role in controlling various types of pathogens.

Jamshidi, N, et al (2017), Concluded that Basil (*O. sanctum* Linn) is the most important herb in Ayurveda, and scientific research of various extracts or specific compounds have been explored *in vitro* and *in vivo* for its health benefits like antimicrobial, antiparasitic, anticancer, antioxidants, anti-inflammatory, anti-hypertensive, anti-hypercholesterolemia, anti-pyretic, antidiabetic, immunomodulatory, anti- allergic, anti-tussive, anti-asthmatic, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritic, adaptogenic, anti-leukodermal and anti-coagulant etc.

N Sing, et.al (2012) reported on the basis of Extensive studies, experimental and clinical proved that Tulsi possess anti-stress/adaptogenic, anti-oxidants, Immuno modulators and anti- radiation properties which also may help it to

play major role in prevention and treatment of cancer. The aim is to stimulate research in this field, prevent and treat human carcinoma by initiating detailed studies in this field. Therefore, it was worthwhile to review its anti-cancer properties to give an overview of its status to scientists both modern and ancient (Ayurvedic).

Pallavi Dixit (2015), found that Tulsi plant had been used by all cultures throughout the history but India has one of the oldest and diverse cultural traditions associated with the use of Tulsi plant. There are numerous uses of Tulsi Plant and it is increasingly finding its way in the ayurvedic treatment of diseases. Tulsi plant has lot of significance for mankind due to its manifold medicinal benefits.

Sai Krishna. G, (2014) Suggested that the Wonder Herb Tulsi is believed to purify expectorants and called the wonder herb. Tulsi is pungent and bitter in taste, pungent in the post-digestive effect and has hot potency. It has a variety of biological; pharmacological activities also it possesses several medicinal properties. Ocimum contains fixed oil which is confirmed by chemical tests for glycerine.

Singh Vishwabhan (2011), Justified that the traditionally different parts (leaves, stem, flower, root and whole plant) of *Ocimum sanctum* herb, native to India, suggested for treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, dermal ailments, ophthalmic problems, insect bites etc. Research shown *Ocimum sanctum* possess anti-fertility, anti-cancer, anti-diabetic, anti-fungal, anti-microbial, hepato- protective, cardio-protective, anti-emetic, anti-spasmodic, analgesic.

2.2 Screening of phytochemicals in Tulsi

The plant parts, such as seeds or grains, flowers, fruits, stems, barks, leaves, and roots, arc used to treat different diseases because of the presence of medicinally important chemical constituents in the and these biologically active or non-nutrient chemical constituents are called phytochemicals or phytoconstituents **(Mousavi et al, 2018)**

Bakkali et al. (2008) found that the unique aromatic odour of basis is because volatile oils are present, which constitutes phenols, terpene, flavonoids and aldehydes. The plant also includes glycosides, alkaloids, tannins, quinones and saponins in addition to an essential oil.

Boarch et al. (2018) isolated proteins, carbohydrates, Phenol, tannin, flavonoids, glycosides, terpenoids, alkaloids after their biochemical test.

Bonjar et al. (2004) revealed that the phytochemical constituent such as alkaloids, steroids, flavonoids, tannins, phenols and several other aromatie compounds of plants serve a defense mechanism against predation by many microorganism, insects and other herbivore.

Bhattacharya et al (2019) found that plant extract of *O.gratissimum, O.tenuiflorum* and *O.basilicum* showed both antioxidant and hypoglycemie effect.

Njoku, O. U, et.al (2011) Antioxidant Properties of *Ocimum gratissimum.* The acute toxicity of the *O. gratissimum* plant extract on mice was resulted to be 2450 mg/kg body weight. Using rat liver homogenate, the antioxidant activities of an ethanol extract of the leaves were tested. When compared to ascorbic acid, ferrous sulphate, nitric oxides, and carbon tetra-chloride induced lipid peroxidation activities showed considerable inhibition at varied concentrations. According to this research, *O. gratissimum* can act as a free radical scavenger, reducing the consequences of free radicals in the body.

Shree, M, et.al (2019), reported that the secondary metabolites were investigated in hot water leaf extracts of, *O. basilicum* and *O. sanctum* by GC-MS. Amino acids (glycine and serine glutamate), sugars and their derivatives (glucose and galactinol), organic and other acids (malie and fumaric acid, succinic acid, 4-amino butanoic acid) and intermediates (trans caffeic acid, ferulic acid, quinic acid, shikimic acid, tartaric and myoinositol) were identified.

2.3 Extraction of essential oil from Ocimum species

According to **Lawrence (1992),** the oil production in India was found to be 15 tons, 5 tons in Egypt, 4.5 tons in Pakistan and the Comoros, 7 tons in Bulgaria, Israel (2 tons) and lower Quantities in Yugoslavia (1 ton), USA (1 ton), Reunion (0.5 ton), Madagascar (1 ton), Albania (0.5 ton), Hungary (0.3 ton), and in Argentina (0.2 ton) According to estimates, from 2019 to 2023, the market for Ocimum species essential oil is expected to increase by $186.5 million, a compound annual growth rate of 8%, with Europe accounting for the biggest market share. Overall, with such large market potential, the essential oil of Ocimum species is a significant source of foreign exchange revenue for developing countries (Gurav, T.P et al 2021)

Abhay Kumar Pandey et al (2014) wrote in his research paper that a variety species of *O. basilicum*, commonly called. African blue basil is known for its camphor like scent of its essential oil. Similarly *O.minimum* and *O. citriodorum* also include variety species of *O.basilicum* popular in India.

Galotto et al (2016) reported that thymol is one of the most common essential oil component obtained principally from thyme species and also from *O. gratissimun* (53.2%)

J. B. Harborne, et al (1993), revealed thatantibacterial/antimicrobial action has been demonstrated in volatile oil. The plant's leaves, stem, and flowers have been found to contain many terpenoid chemicals. Some of the chemicals

are utilized as antifungals, such as thymol, linalool, and carvacrol, while camphor and camphene are being used as topical antipruritics.

Khalid. K.A, et al (2006) concluded that a high degree of poly morphism in the genus Ocimum determines a large number of sub species, different varieties and from producing essential oil with varying chemical composition offering variable level of meidicinal potential.

Maria Loredana Soran et al (2009) was isolated α and β Pinene, (Mixture) Limonene, Citro nellol, and geraniol using the solvent ethyl ether & ethanol in the ratio of 1:1 by TLC

Pandey A.K et al (2014) isolated Linalool, 1,8-cineol, eugenol, pinene, methyl chavicol, camphor, ocimene, limonene and terpinene are among the main chemicals from several Ocimum species.

Sadaf Quereshi & et al (2011) isolated the extraction of oil from three different Ocimum species viz-*O. gratissimum, O. sanctum* and *O. americanum* were carried out with oil yield of 1.0%, 1.5% and 1.25% respectively.

Tchatchouang et al (2017) isolated that the main constituent present in the essential oil of African (Cameroon & Rwanda) Ocimum, gratissimum are β-Phellandrene, γ- terprinene, limoneme, p-cymene, thymol and eugenol

2.4 Chromatography technique.

Chromatography is a technique employed for the separation of chemically closed substances into individual component by their partition co-efficient (Jahanger et al 2022)

Norr, H & Wagner, H (1992) isolatedthe existence of the phenolic compounds gallic acid ethyl & methyl ester, protocatechuic acid, vanillin, 4-hydroxybenzoic acid, and 4-hydroxybenaldehyde was verified using high-performance liquid chromatography (HPLC)

Quach et al (2004) extracted and separated the leaf Pigments by thin layer chromatography. They used TLC techniques for the separation of number of colorful Pigment from the leaves.

Sadaf Qureshi et al. (2011) isolated plant pigments such as chlorophyll-a, chlorophyll-b, Xanthophyll and carotene through TLC method and R_f Value was then calculated.

2.5 Molecular characterization of Ocimum species related review paper.

Ahamad Bilal (2020), reported that theGel electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their size and charge. Electrophoresis involves running a current through a gel containing the molecules of interest. Based on their size and charge, tne molecules will travel through the gel in different directions or at different speeds, allowing them to be separated from one another.

Khanujaet at. (1999) carried out rapid isolation of DNA from dry and fresh samples of plant producing oils. Plant species belonging to the same or related genera can exhibit enormous variability in the complexity of pathways of dispensable function. Thus the biochemical composition in plant tissue of different species is expected to vary considerably. They extracted DNA from *O.kilmandelens* and carried out PCR amplification.

Matasyoh, et al (2008) isolated genomic DNA from the *O. gratissmum* by CTAB method, the DNA was quantified and examined for intactness using the gel electrophoresis method (Qiagen, 1997), the yield of DNA per gram of leaf tissues extracted was also quantified using a Bio-photometer at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm (Sambrook et al 2001)

Rastogi et al 2015 & upadhyay et al (2015) reported that some metabolites affects the quality DNA isolated *O. sanctum*. At present, commercially available kits for DNA isolation or purifications seems the only source for

high quality DNA purification. This is also evident in the genomic DNA isolation methodologies used for *O. sanctum* in recent report.

Singh Charan, et al (2015) concluded that a phytochemical and DNA quantification level of Tulsi and Shyama Tulsi bands found at same base pair, phytochemical test are same whereas van Tulsi & Shyama Tusli show same result. Therefore, it can be said that van Tulsi & Shyama Tulsi show so many similarities.

2.6 Sanger Sequencing

Aleena Alex et al (2020) concluded that the Sanger process has made it possible for researchers to sequence stretches of DNA at speeds never before possible. It has increased the sequencing rates drastically cut the cost of sequencing and may eventually allow every person the possibility of personalized genomic information. It also offers the promise of advanced medical treatments, which require a considerable amount of work to generate, understand, organize, and apply this massive amount of data to human disease.

Chansheng Li, et al (2017) reported that Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain terminating dideoxy nucleotides in the process of DNA amplification.

Mohammad Baqur Al Shuhaib & Hayder O. Hashim (2023) Concluded that Sanger sequencing is a widely employed method for DNA sequencing, entails reading DNA sequences from chromatograms generated during sequencing. Nonetheless, interpreting DNA chromatograms can prove challenging in many clinical applications due to a range of technical intricacies that may result in misinterpretation of sequences. Among these complexities are noise, artifacts, and confusion between heterozygosis and double infection potentials, along with other errors. Thus, technical aspects, serving as a resource for researchers and analysts striving to enhance the precision of DNA sequencing results and bolster downstream analyses.

Smith L.M & Kaiser RJ et al (1986) developed a method for the partial automation of DNA sequence analysis. Fluorescence detection of the DNA fragments is accomplished by means of a flurophore covertly attached to the oligonucleotide primer used in enzymatic DNA sequence analysis. A different coloured flurophore is used for each of the reactions specific for the bases A, C, G & T. The reaction mixtures are combined and co-electrophoresed down a single polyacrylamide gel tube, the spirited fluorescent bands of DNA are detected near the bottom of the tube, and the sequence information is acquired directly by computer.

2.7 Traditional use

In regarding of application of ocimum species (Tulsi), it was seen that a diverse range of commercially important phytochemical are being extracted from pants. Ancient scholastic work such as the Atharveda, Ayurveda, Unani system and many more included a wealth of knowledge on preventive and therapeutic remedies from plant source (Aggarwal 2015 & Jahanger 2024)

Ahmad N, (2002) & P. Pattanayak, (2010) reported that Benzene extract of *O. sanctum*. Leaves have a reversible antifertility effect, as *O.sanctum* L.extract (250 mg/kg body weight) for 48 days decreases the total sperm count, sperm motility and forward velocity. The percentage of abnormal sperm increased in caudal epididymis fluid and the fructose content decreased in the caudal plasma of the epididymis and the seminal vesicles. All these parameters returned to normal two week after the withdrawal of the treatment.

Borah & Biswas, (2018) concluded the presence of various bioactive compounds in Tulsi leaves, justifies the uses for various ailments by living population. The results confirm the use of *Ocimum sanctum* plant as traditional medicinal properties.

Chowdhary, T et al (2017) reported that the fresh leaf of *O. tenuiflorum* is used in the treatment of common colds, fevers, inflammation and diabetes

just like other ocimum species, and root extract is utilized as a sexual stimulant in various parts of West Bengal, India.

Shokeen P, et al (2005) reported that the extract of *O.sanctum* caused inhibition of Neisseria gonorrhoeae clinical isolated and WHO organization strains. The activity is comparable to penicillin and ciprofloxacin.

CHAPTER-3

Materials & Method

3. Materials & Methods

3.1 Collection and identification of selected plant.

For collection of selected plant the following area of Munger has been visited.

(i) Forest of Bheem Bandh - It is away 58 kms from Munger Rly. Station in South West

(ii) Rishi Kund - It is away 20 kms from Munger Rly. Station in East

(iii) Biotech Nursery - It is away 4 kms from Munger Rly. Station in South

(iv) Jamalpur Hill - It is away 10 kms from Munger Rly. Station in South

(v) Peer Pahar - It is away 4 Kms from Munger Rly. Station in in East

(vi) Area of Chandi Asthan - It is away 4 kms from Munger Rly. Station in North

(vii) Botanical Garden of R. D. & D. J. College, Munger - It is away 3 kms from Munger Rly. Station in South

(viii) Red Fort Area of Meer Quasim - It is away from 2 kms from Munger Rly. Station in West

(ix) Krishi Vigyan Kendra - It is away from 4 kms from Munger Rly. Station In East

(x) Kharagpur Lake - It is away from 45kms from Munger Rly. Station in South

Out of the above famous area, plant are mainly selected from the following places.

(i) Forest of Bheem Bandh

(ii) Fort Area of Munger

(iii) Krishi Vigyan Kendra (KVK) Campus

(i) Forest of Bheem Bandh: The area is famous for its hot springs and deep forest. The Sanctuary is spread across in area of 682 Sq. km. It is believed that Bheema, one of Pandavas from Mahabharata. Once constructed a Bandh (Dam) here and, hence the place is called Bheem Bandh. Bheembandh wild life sanctuary is a Wild life sanctuary in Bihar in the South West of Munger District. It is away from 58 Km approx from Munger Railway Station.

(ii) Fort Area: The Munger Fort, located at Munger in the state of Bihar, it is built by Mohhemadan Kings of India. It is situated in main town of Munger and away from 2km from Munger Railway Station.

(iii) Krishi Vigyan Kendra Campus: Krishi Vigyan Kendra, Munger has been established in April 1979, it is a unit of ICAR. It is away from 4 kms from Munger Railway Station.

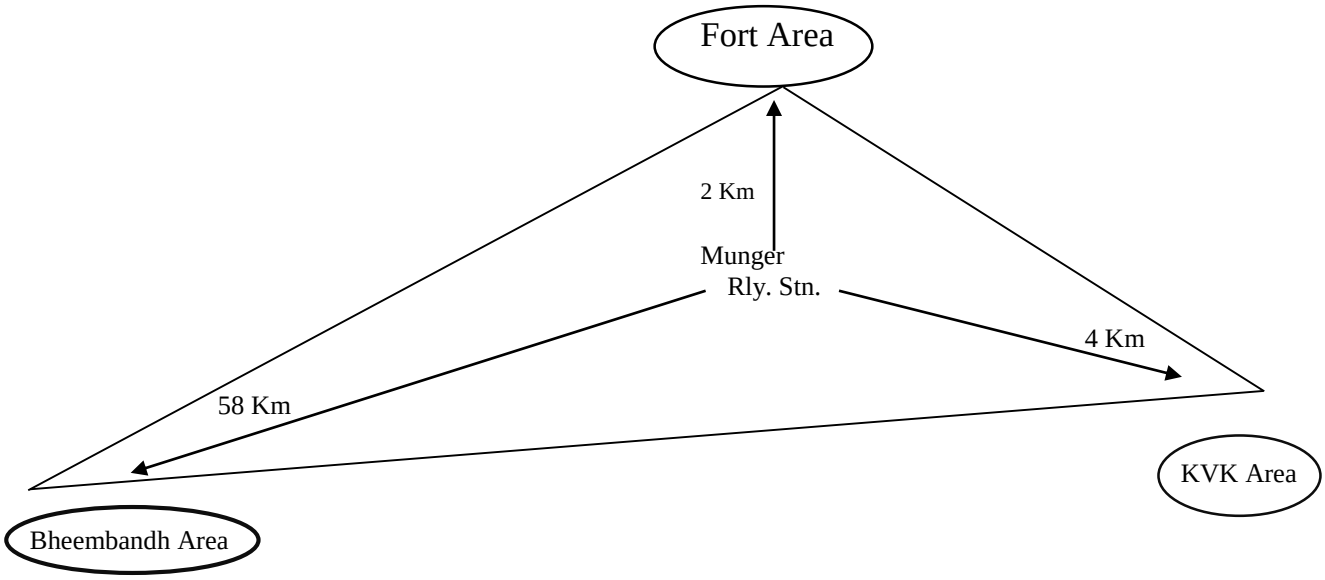


Figure 3.1: Map showing the study area of Munger District, Bihar (India)

→ There are more than 10 species of Ocimum in India but selected three species like *O.tenuiflorum* L., *O.basilicum* L. and *O. gratissimum* L. have been selected because these species are found in the area of Munger District.

→ *O. tenuiflorum* L. are found in every places including Bheem Bandh area, Fort Area and KVK campus. Whereas *O. basilicum* L. is found in Bheam

Bandh area and *O. gratissmum* L. are found in both Bheem bandh and KVK Campus.

3.2 Physical and chemical characteristics of the soil analyzed from the area under investigation: - Rhizosphere soil was analyzed of Fort area, KVK area and Bheembandh area with their Soil texture, Colour and pH Value.

3.2.1 Soil Texture: -The concept of soil texture was first developed by Milton Whitney in 1911 soil texture, a critical soil property is determined by the proportion of sands, Loamy or silt & clay particles. These proportion influence various soil characteristic like water holding capacity, aeration and each of work ability.

Method: - The method to determine the texture of soil was used "Hand texture method" this method involves feeling a small sample of soil to assess its texture by forming squeezing it into a ball.

3.2.2 Soil Colour: - Soil colour is important because it provides clues about the soils composition, fertility, and drainage and moisture retention. Soil colour is a valuable indicator of soil properties and can provided insight into the soils potential for supporting plant life and other ecological functions.

Method: - This test was developed by Albert Henry Munsell in 1918. In the form of colour disc. This disc of colour chart is divided into five principal hues: Red, Yelow, Green, Blue and Purple along with 5 intermediate hues ie YR (Yellow-Red), GY (Green-Yellow), BG (Blue-Green) & RP (Red-Purple)

Soil was taken in petridishes and spreaded. Now soil colour was matched with Munsell colour disc and result was noted in table No.4.1

3.2.3 Soil pH: - pH is potenz of Hydrogen, Potenz is German word means power ie Power of hydrogen. The pH scale is developed by Sorenson. Soil

pH refers to the measures of soil acidity or alkalinity expressed on a pH scale. A pH below than 7, indicates acidity, while a pH above 7, indicates alkalinity and pH is equal to 7, indicates neutral.

Method: - 10 gms. Soil sample was collected from rhizosphere of Ocimum species and dissolved in 100 ml D.W. Pen type pH meter was into soil solution and reading was noted in table no-4.1.

This process was repeated separately for all three selected places

→ There are More than 10 species of Ocimum in India but selected three species like *O.tenuiflorum* L., *O.basilicum* L. and *O. gratissimum* L. have been selected because these species are found in the area of Munger District.

→ *O. tenuiflorum* L. is found in every places including Bheem Bandh area, Fort Area and KVK campus. Whereas *O. basilicum* L. is found in Bheem Bandh area and *O. gratissmum* L. are found in both Bheem bandh and KVK Campus.

3.3 Identification of Species: -After collection of Ocimum species, it was identified & differentiated among three species on the basis of morphological characters like plant height, leaf size and leaf colour as in table no. 4.2

3.4 Phytochemical screening of Ocimum species (Qualitative)

For phytochemical analysis as qualitative, following materials are required. Methods, used for the analysis of primary and secondary metabolites is also supported by Borah R & Biswas S.P, 2018 who was also found protein, sugar, alkaloid , flavonoid, glycoside, terpenoid, Phenol & steroids.

Materials

Apparatus: - Test tube, Test tube holder, petridishes, beaker, Pipette, spirit lamp and digital balance.

Chemicals: -Biuret reagent, Benedict reagent, Millon's reagent, Fehling solution (A & B), HCL, Mayer's reagent, Magnesium, 2% NaOH,

Chloroform, Conc. H_2SO_4, Acetic Acid, Ethyl alcohol, Ferric chloride & Distilled water.

3.4.1 Plant Meterials collection &Preparation of plant extract: -Fresh leaf samples from healthy, mature plants of each of the three Ocimum species were collected from the three designated locations,The collected leaf samples were immediately placed in sterile polythene bags, labeled with the species name, collection location, and date of collection, and transported to the laboratory. Upon arrival, the leaf samples were washed thoroughly with distilled water to remove any surface contaminants and then air-dried under shade at room temperature for several days until a constant weight was achieved. The dried leaf samples were grinded into a fine powder using an electric grinder and stored in airtight containers at room temperature, protected from light and moisture, until further analysis.

5 grams of fresh leaves of all the three species has been collected and kept separately in three petridishes, macerated in D.W and centrifuged. After centrifuge, extract was collected and kept in three separate vials and symbolized it as *O.tenuiflorum* L., *O.basilicum* L. and *O.gratissmum* L..

3.4.2 Analysis of primary & secondary metabolites

In plant leaves, primary metabolites like carbohydrates, lipids and proteins are essential for growth and development. Whenever, secondary metabolites play crucial roles in plant defame, adaptation and other physiological function. Example of secondary metabolites in plant leaves are Alkaloids, Flavonoids, Glycosides, Terpenoids, Phenols and Steroids. Tulsi has been used in traditional sustems like Ayurveda and Unani for Centuries. Scientific study of secondary metabolies helps explain why Tulsi shows antimicrobial, anti inflammatory, anti oxidant and adaptogenic effect. This bridges traditional knowledge with modern science.

3.4.2.1Test of Protein: -

1. **Biuret Test:** The Biuret test for protein was first documented by the physiologist Gustav Potroski in 1857. In this test, 2 ml of extract was taken

in a test tube and 2 ml Biuret reagent was added. It was mixed and heated through spirit lamp.

2. **Millon's Test: -**This test was discovered by the French chemist Auguste Nicolas Eugene Millon in 19th Century. In this test, 2 ml plant extract was taken in a test tube and 4 ml Millon's reagent was added. It was mixed and heated through spirit lamp.

3.4.2.2 Test of Carbohydrates: -

1. **By Fehling Solution: -**This test is developed by the German chemist Hermann Von Fehling in 1849 to identify the presence of reducing sugers like Glucose. Fehling solution is a chemical reagent consisting of two solution (A&B) that are mixed. In this test, 2 ml plant extract was taken in a test tube and 1 ml each of Fehling solution A & B where added. It was mixed and heated.
2. **By Benedict test: -** This test is developed by American chemist Stanley Rossitev Benedict in 1908. When a sample containing reducing sugar like glucose, is heated with Benedicts reagent the copper (II) ions reduced to copper (I) ion, gives brick red colour. In this test, 2 ml plant extract was taken in a test tube and 2 ml of Benedict reagent was added. It was mixed and heated.

3.4.2.3 Test of Alkaloid: -

1. **By Mayer's reagent: -** This test is developed by the German chemist Julius Robert Von Mayer in 19th Century. In this test, 2 ml plant extract was taken in a test tube and 2 ml 1% HCl is added. It was mixed and heated then added Mayer's reagent.
2. **By Wagner's reagent: -** This test developed by Wagner in 1886. Inthis test, 2 ml plant extract was taken in a test tube and 2 ml 1% HCl is added. It was mixed and heated then added Wagner's reagent.

3.4.2.4 Test of Flavonoids: -

1. **Shinoda test: -** This test is developed by Japanese chemist Toshio Shinoda in 1939. In this test, 2 ml plant extract was taken in a test tube and little amount of magnesium and conc. HCl was added drop wise.

2. **By NaOH Solution: -** This test is developed by Trease and Evans in 1950. In this test, 5 ml plant extract was taken in a test tube and 2 ml of 2% NaOH solution was added.

3.4.2.5 Test of Glycosides: -

1. **Keller Killiani test: -** This test is first developed by Hans Keller and Otto Killiani in 20th Century. In this test, 2 ml plant extract was taken in a test tube and 2 ml chloroform +2 ml acetic acid were added and it was mixed and cooled. Lastly conc H_2SO_4 was added.

2. **Salkowsky test: -** This test is developed by Earnst Salkowsky in 19th Century. In this test, 2 ml plant extract was taken in a test tube and 2 ml chloroform +2 ml conc H_2SO_4 were added. Shaked gently.

3.4.2.6 Test of Terpenoid: -

Salkowsky test: - This test is developed by Earnst Salkowsky in 19thCentury. In this test, 2 ml plant extract was taken in a test tube and 2 ml chloroform added and warmed till dryness. Now 2 ml conc H_2SO_4 and it was heated three minutes.

3.4.2.7 Test of Phenol: -

Ferric Chloride test: - This test was first developed by Fridlieb Ferdinand Runge in 1834. In this test, 2 ml ethyl alcohol was taken in a test tube and 2 drops ferric chloride was added, then 1 ml of plant extract was added.

3.4.2.8 Test of Steroids: -

Salkowsky test: - This test is developed by Earnst Salkowsky in 19th Century. In this test, 2 ml plant extract was taken in a test tube and 2 ml chloroform was added. It was mixed and then 2ml conc. H_2SO_4 was added.

3.5 Preparation of standard curve of Protein by Lowry method: -

In 1951 H. Lowry and his colleagues developed a methods to draw standard curve from standard protein like Bovine serum albumin (BSA) and has since

become one of the most widely used protein assays due to its high sensitivity, accuracy and reproducibility.

Materials:

A. Equipments: Test tubes, beaker, measuring cylinder, pipettes, torsion balance, colorimeter, cyclomixer

B. Reagents:

1. Bovine albumin (0.1mg/ ml)
2. $CuSO_4$ reagent
   - a/ 2% Na_2CO_3
   - b/ 0.1 (N) NaOH
   - c/ 0.5% $CuSO_4$ soln.
   - d/ 1% Na-K tartarate
3. 30% TCA (Tri Carboxylic acid)
4. F.C. reagent – 1 (N)

Method:

A. Preparation of reagents:

1. Bovine albumin (0.1mg/ml)

   10mg bovine albumin is dissolved in 100ml D.W.
2. $CuSO_4$ reagent

   a/ 2% Na_2CO_3

   2gm $Na_2 CO_3$ is dissolved in 100ml D.W.

   b/ 0.1(N) NaOH

   40gm NaOH dissolved in 1000ml D.W. for 1N

   4gm NaOH dissolved in 1000ml D.W. for 0.1N

   c/ 0.5% $CuSO_4$ soln.

   500mg $CuSO_4$ is dissolved in 100 ml D.W.

   d/ 1% Na- K tartarate

   1gm Na-K tartarate is dissolved in 100ml D.W.

Soln. (A) – 2% Na_2CO_3 dissolved in 0.1 (N) NaOH

Soln. (B) – 0.5% $CuSO_4$ soln.

Soln. (C) – 1% Na-K tartarate

100 ml of sol^n. (A)

1ml of sol^n. (B)

1ml of sol^n. (C) .

102ml of $CuSO_4$ reagent

3. 30% TCA

30gm TCA is dissolved in 100 ml D.W.

4. 1(N) F.C. reagent

Preparation of standard curve: -

11 dry, clean test tubes was taken and marked them 1 to 10 and 'B' (for Blank).Different protein conc was taken in test tubes marked 1 to 10 (10ug to 100ug), by adding 0.1 to 1.0ml of working protein soln. respectively.Made the vol. 1.0 ml by adding required amount of D.W. i.e. from 0.9 to 0.0ml in T.T marked 1 to 10 respectively. Take 1.0ml D.W. in B.Added 5.0 ml $CuSO_4$ reagent in each test tube. (Wait for 10 min.). Added 0.5ml F.C. reagent in each test tubeCyclomixed on a cyclomixer (Wait for 10 min.). Read on colorimeter at 620nm, Now Plotted the graph, conc. of protein on X-axis & respective O.D on Y-axis. Values are tabulated on table no-7

3.5.1 Quantitative analysis of Protein in selected species of Ocimum Species using standard curve (Lowry et al 1951).

For quantitative analysis of Protein, extract was prepared and kept in three separate vials and labelled it as below:

O. tenuiflorum L.	vial No. – 1	as O.t
O. basilicum L.	vial No. – 2	as O.b
O. gratissmum L.	vial No. – 3	as O.g

Tulsi extract (100mg/ml) was prepared by homogenizing of 2 grams Tulsi leaf in 20ml D.W. and similarly made separate vials for O.t, O.b and O.g.

Three centrifuge tubes were arranged on test tube stand and written as O.s, O.t., O.b. and O.g in test tube no. 1, 2 and 3 respectively. 0.5ml homogenate from each vials was transferred in test tube no. 1, 2 and 3respectively. 2.0 ml of 30% TCA was added in each test tubes. Left for one hour. Centrifuged for 20min. at 2000rpm, supernetant was discarded.Left for 1hr. in inverted position. 2.0ml 1(N) NaOH was added& Shaked gently. Protein extract prepared. 0.5ml extract from each test tube has transferred into another test tubes, labelled it and arranged on test tube stand. Made the volume 1.0ml by adding 0.5ml D.W in each test tube, Fourth test tube 'C' was also arranged for control and put 1.0ml D.W.Made the volume 1.0ml by adding 0.5 ml D.W in each test tube. Added 5.0ml $CuSO_4$ reagent in each test tube.Wait for 10 min.Added 0.5ml F.C. reagent in each test tube cyclomixed on cyclomixer. Wait for 30 min.Read on colorimeter at 620nm.

3.6 Preparation of standard curve of Glucose by Nelson's method.

A standard curve of glucose is a graph that shows the relationship between the concentration of glucose and the absorbance of light passing through glucose solution. This method is given by Nelson, in 1944. Due to accuracy and sensitivity, this method is widely used to determine the concentration of glucose from unknown sample.

Materials:

A. **Apparatus/Equipments:** Test tube, Beaker, Measuring Cylinder, Pipettes, Water bath, Colorimeter, Cyclomixer.

B. **Reagents/Chemicals:** Glucose stock solution, Nelson's reagent, Arsenomolybdate reagent.

Preparation of reagents:

→ **Glucose stock solution:** (1mg/ml)

100mg standard D-glucose was dissolved in 100ml D.W.

→ **Glucose working solution** (100µg/ml)

1ml stock solution dissolved in 9ml D.W.

→ **Nelson's Reagent**

Solution A:	Na_2CO_3	-	12.5 gms.
	Na-K Tartarate	-	12.5 gms.
	$NaHCO_3$	-	10.0 gms.
	Na_2SO_4	-	100 gms
	[Dissolved in 500ml D.W.]		

Solution B: $CuSO_4.5H_2O$ – 7.5gram & 1 drop of concentration H_2SO_4 dissolved in 50ml D.W.

Sol^n. A	25ml
Sol^n. B	1ml
	26ml – Nelson's reagent is ready to work

Method:

11 clean and dry test tubes were arranged on test tube stand and marked as B, 1, 2, 3 …10.Now0ml, 1ml, 2ml, 3ml …. 10ml Glucose working solution was poured in test tube No. B, 1, 2 …10 respectively, then1.0 ml, 0.9ml ….0.0 ml D.W. was added in test tube No. B, 1, 2 …..10 respectively. 1.0ml Nelson's reagent was added in each test tube. Warmed at water bath for 20min at 100^0C. Left for cool. 1.0ml Arsenomolybdate reagent was added in each test tube& cyclomixed. 7.0 ml distilled water was added in each test tube. %T & O.D. were recorded through colorimeter at 540nm. Colorimetric reading was noted Table No. 4.7

3.6.1 Quantitative analysis of sugar in Ocimum species using standard curve (Nelson et al 1944):

Extract (100mg/ml) was prepared by homogenizing 2 gram Tulsi leaf in 20ml D.W,centrifuged and supernetant was collected from centrifuge tube for three selected Ocimus species and marked as o.t, o.b & o.g. 1ml 10% $ZnSO_4$ was added in all three test tube. 1ml 0.1 (N) NaOH was added for precipitation

occurring. Left for 30 min. Centrifuged at 2000 rpm for 20 min.Supernetant was collected from each centrifuge tube.Now all test tubes were arranged on test tube stand and fourth test tube marked as 'C' (For control) O.t, O.b and O.g in test tube No. 1, 2, 3 and 4 respectively. 0.1 ml supernetant was taken in each test tube no. 1, 2, and 3 & 0ml in test tube 4 (Control). 0.9ml D.W was added in test tube No. 1, 2, 3 and 4 so the whole vol. become 1ml and 1ml only D.W was taken in test tube No. 4 (Control). 1 ml Nelson's reagent was added in each test tube. Warmed on water bath at 100^0C for 20 min. Cooled under running tap water. 1ml Arsenomolybdate was added in each test tube. Cyclomixed and 7ml D.W was added in each test tube and again cyclomixed. Colorimeter reading was taken out and it was tabulated in Table No. 4.8.

3.7 Quantitative analysis of Alkaloids in Ocimum species by Gravimetric method: -

The gravimetric method for analysis of alkaloids in Ocimum species was developed by J.B. Harbone in 1973.

Materials:

- **A.** Appartus/Equipments: Test tubes, Beaker, Measuring cylinder, Pipettes, Balance, Cyclomixer, dessicator, Whatmann Paper.
- **B.** Chemical/Reagents: Ethanol, Acetic acid, Ammonia
- **C.** Leaves of four selected species of Ocimum.

Method:

To determine TAC (Total Alkaloids Content) 5 grams of selected Tulsi leaf was grinded and soaked in 20 ml of 10% Ethanolic Acetic Acid. Left it 4 hrs. At room temp. After it mixture was filtered through Whatman paper. The filterate is concentrated by evaporation over a steam bath till the original volume become $1/4^{th}$. Ammonia solution was added drop wise till yellow ppt. Now this ppt. was filtered using previously weight filter paper. After filtration, the ppt. was cooled in dessicator. Now filter paper was again

weighed. The above process was repeated upto 3 times for all the three selected Tulsi Leaves separately. Observations were recorded in Table No. 4.10, 4.11, & 4.12

3.8 Preparation of standard curve of Flavonoids by Aluminum Chloride method (Christ & Muller, 1960): -

The Aluminum chloride colorimetric for the preparation of standard curve of flavonoids through quarcetin was developed by Christ & Muller in the year of 1960, for analysis of flavanol derivatives in drugs.

Materials:

A. Appartus/Equipments: Test tubes, Test tube stand, Pipette, Measuring cylinder, Beaker, Balance Cyclomixer

B. Chemical/Reagents: Quarcetin, 10% Ammonium Chloride, 1-M Sodium acetate, D.W. Methanol

Prepration of reagent

→ Preparation of 10% Quarcetin reagent (1mg/ml) 25mg quarcetin dissolved in 25 ml of methanol.

→ 10% Aluminum Chloride: 5gms. Aluminum chloride dissolved in 50ml of D.W

→ 1M – Sodium acetate: 82gms. Sod. Acetate dissolved in 1000ml DW. 4.10gms. Sod. Acetate dissolved in 50ml DW.

Methods

11 dry and clean Test tubes were arranged on test tube stand and marked as B, 1,2, 3 …10 respectively0ml, 0.1ml, 0.2ml – 1.0ml of quarcetin reagent was taken in T.T. No. B, 1, 2 ….10 respectively. 1 ml, 0.9ml, 0.8ml … 0.0ml methanol was added in T.T.No. B, 1, 2,…….10 respectively. 2ml of 10% $AlCl_3$ was added in each test tube and Cyclomixed. Colorimeter reading was taken out at 415nm of each tube. Colorimetric reading was redorded in Table No. 4.15.

3.8.1Quantitative analysis of flavonoids in Ocimum species using standard curve (Christ & Muller, 1960):

Four dry & clean test tubes was taken and marked as O.t, O.b, O.g and B (B for Blank) 2 ml leaf extract from each species was poured in each tubes separately, avoided contamination, 2 ml $AlCl_3$ was added in all the four tubes. It was cyclomixed. Colorimeter reading was taken at 415 nm of each tube and it was tabulated in table no-4.16.

3.9 Preparation of standard curve of Terpenoids (Ghorail et al 2010.)

A standard curve is a graph that plots the concentration of a known standard solution against it absorbance i.e, optical density. For total terpenoid content using a compound like Linalool. Linalool is a common monoterpene.

Materials

A. Apparatus/Equipment: - Test tubes, Test tube stand, pipette, measuring cylinder, Beaker, Balance, cyclomixer, colorimeter.
B. Chemical/Reagents: - Linalool, Chloroform, 95% Methanol, Conc. H_2SO_4, D.W

Methods

11 dry and clean test tubes was arranged on test tube stand and marked as B,1,2,3……10 respectively. 0 ml, 0.1 ml, 0.2 ml, …….1.0 ml Linalool solution was transferred in test tube no B,1,2,3…….10 respectively by means of pipette. 2.0, 1.9, 1.8..1.0 ml chloroform was added to test tube no. B,1,2,3….10 respectively. Left 10 min for incubation reddish brown colour was obtained.Now 1.5 ml 95% methanol was added in each test tube. It was cyclomixed for each tube. Now solution was transferred into cuvette to take colorimetric reading at 540 nm. Colorimetric observations were recorded in Table No. 4.17.

3.9.1Quantitative analysis of terpenoids in all the four species of Ocimum using standard curve (Ghorail et al 2010.)

3 test tube was arranged on test tube stand and marked as O.t, O.b, and O.g.

2 ml extract was transferred in test tube no O.t, O.b and O.g respectively. 1.5 ml chloroform was added in each test tube. Left for 10 minutes. Now 1.5 ml 95% methanol was added in each test tube. Cyclomixed and colorimeter reading was noted at 540 nm. This reading was coincided with standard curve (Fig. No. 16) and amount of terpenoids was determined.

3.10 Preparation of standard curve of Gallic acid for estimation of Phenol (Nazish Siddique et al 2017)

A standard curve is a graph that plots the concentration of a known standard solution of Gallic acid against its absorbance using spectrophotometer.

Materials:

A. Apparatus/Equipments: - Test Tubes, Test tube stand, Pipette, Measuring cylinder, Beaker, Balance, Cyclomixer, Spectrophotometer.

B. Chemical/Reagent: - Gallic acid, Methanol, Sodium carbonate, Distilled water, F.C reagent.

Prepartion of Reagent: -

10 mg Gallic acid dissolved in 10 ml of methanol, followed the concentration of 1 mg/ml

Method: -

11 dry and clean test tubes was arranged on test tube stand and marked as B,1,2,3……10 respectively. 0 ml, 0.1 ml, 0.2 ml, …….1.0 ml Gallic acid solution was transferred in test tube no B,1,2,3…….10 respectively by means of pipette. 2.0, 1.9, 1.8..1.0 ml D.W was added to test tube no. B,1,2,3….10 respectively, the volume become 2 ml in each tube. Now 2ml F.C reagent was added in each tube, mixed well though cyclomixer and Lastly 1 ml sodium carbonate was added in each tube so the whole volume become 5 ml in each tube, cyclomixed each tube.

Now solution was transfered into cuvette in appropriate amount to take spectrophotometric reading at 765 nm. Reading was tabulated in Table no. 4.20.

3.10.1 Quantitative analysis of phenol in all the four species of Ocimum using standard curve (Supported by Renu Pandey et al 2015 & Nazish Siddique et al 2017)

Three test tubes was arrange on test tube stand, marked as O.t, O.b and O.g. 2 ml plant extract was transferred in each test tube. 2 ml D.W was added and then 1 ml F.C reagent was added, left for 10 minutes. Now 2 ml sodium carbonate was also added in each test tube. Cyclomixed and spectrophotometric reading was taken for each tube separately at 765 ml. This reading was coincided with standard curve and amount of phenol was determined, as in Table no. 4.20

3.11 Prepration of standard curve of Digitoxin for estimation of Glycosides:

A standard curve of Digitoxin is a graph that shows the relationship between the concentration of Digitoxin and the absorbance light passing through cuvette containing Digitoxin soluation this method also supported by Vaishali Patil et al 2023**.** Due to accuracy and sensitivity, this method is widely used for determination of cardiac glycosides. Cardiac glycosides are a class of drugs, also known as cardiotonics that primarily effect the heart.

Materials: -

A. **Apparatus/Equipments:** Test tubes, Beaker, Measuring cylinder, Pipette, Volumetric flask, Spectrophotometer, cyclomixer.
B. **Reagent/Chemicals:** Digitoxin, Baljet reagent, Ethyl alcohol, Distilled water.

Preparation of reagent/solution:

→ **Stock solution of Digitoxin:** 100 mg standard digitoxin powder was dissolved in 100 ml of volumetric flask containing ethyl alcohol to get 1 mg/ml solution

→ **Working solution of Digitoxin:** 1 ml stock solution of digitoxin dissolved in 9ml of D.W to get 100 μg/ml

Method:

11 clean and dry test tube were arranged on test tube stand and marked as B,1,2,3…..10. Now 0 ml, 0.1 ml, 0.2 ml …….1.0 ml Digitoxin working solution was poured in Test tube No. B,1,2…….10 respectively. Now 1.0 ml. 0.9 ml, 0.8…….0.0 ml Baljet reagent was added in test tube no. B,1,2,3,……10 respectively. Left for 5 minutes and spectrophotometric reading was taken as 220 nm. All observations were recorded in Table No. 4.21.

3.11.1 Quantitative analysis of cardiac glycosides in Ocimum species:

Four dry and clean test tubes was taken and marked as O.t, O.b, O.g and B for blank. 2 ml leaf extract from each species was poured in each tube separately, avoided contamination. 2 ml Baljet reagent was added in each tube. It was cyclomixed. Spectrophotometric reading was taken at 220 nm. Observation was tabulated in Table No. 4.22

3.12 Analysis of Plant Pigments by paper chromatography technique (M. Tswette, 1903)

Chromatography technique is developed by Mikhail Tswette in 1903. It is technique employed for the separation of chemically closed substances into Individual component by their partition co-efficient. Following are two ways for this test.

3.12.1 Qualitative Test: - Qualitative analysis of plant pigments from leaf extracts involves separating and identifying different pigments present in leaf sample.

Materials:

Mortar & Pestle, Glass Jar, Measuring cylinder, Beaker, Whatsman Paper No. 1

Reagents: 1. Homogenate of Leaves
2. 80% acetone
3. Solvent system (Petroleum ether + Methanol (49ml + 1ml)

Method:

[a] Preparation of homogenate :

Soft part of Ocimum leaf was cut with small pieces and homogenized with clean sand with the help of Mortar & Pestle, 80% acetone was added drop wise so that pigments dissolved in it.

[b] Preparation of Solvent: Solvent was prepared by mixing 49 ml Petroleum ether and 1.0 ml methyl alcohol

[c] Methodology: Whatman Paper No. 1 of appropriate size was cut and a line was drawn through pencil after 2cm of one edge. Three (3) Spot was made as small circle and written as O.t, O.b & O.g. Homogenized sample was loaded & left to dry. Now loaded Paper was transferred into Jar contains solvent upto 1cm from the bottom. It was left to run the solvent upto 10 cm.

3.12.2 Quantitative Test: - Quantitative analysis of plant pigments from leaf extract typically involves extracting pigments with a solvent like acetone followed by spectrophotometric measurement to determine the concentration of chlorophyll a, chlorophyll b, carotene and xanthophyll.

Materials & Method:

Apparatus/Equipments: Spectrophotometer, Volumetric flask (100ml), Mortar & Pestle, Test tube & Funnel.

Chemical/Reagents: 80% acetone, Homogenate

[a] Preparation of homogenate

5 gm of Ocimum Leaf was taken and homogenized in mortar & Pestle with sand and 80% acetone.

[b] Procedure:

The Whole amount of homogenate was transferred into 50ml volumetric flask with. the help of funnel. The vol. of homogenate maintained 50ml by addition of 80% acetone.It was kept for whole night in dark.Next day the extract was collected in another test tube. Reading was taken at 660nm and 620nm against blank of 80% acetone. The above experiment is done separately for following species of Ocimum (Tulsi)

(a) *O. tenuiflorum* L. (b) *O. basilicum* L. (c) *O. gratissimum* L.

3.13 Oil Extraction through Clevenger apparatus (J.F. Clevenger, 1928):

The Clevenger apparatus works by heating the sample, causing the volatile organic compounds to vaporize and be carried away by the vapour. The condenser then cool the vapour, causing it to condense into a purified form, which was collected in the collection flask. The Clevenger apparatus is named after its inventor Joseph Franklil Clevenger.

Essential oil are good source of several bio active compounds which possess antimicrobial, anti-inflammatory and anticancerous properties. The main constituents present in the essential oil of *O.gratissimum* L. are Phellandrene, Terpinene, Limonene, Thymol and Eugenol (Tchatchoung et al 2017). The major chemical components present in the essential oil of *O.basilicum* L. are 1, 8-cineole, Linalool, Methyl chavicol, Eugenol, Methyl cinnamate,

Thymol, Limonene **(**Pandey A.K et al 2014**).** Thymol is one of the most important common essential oil obtained from *O.gratissimum* L. (Galotto, M.J et al 2016).

Materials:

Leaves of Ocimum species, D.W, Clevenger apparatus, Mortar& Pestle

Method:

30 gms. Crushed leaves of Ocimum species has taken in Clevenger flask**.** 300 ml D.W was added**.** Heated 4 hrs.Oils separated and it was collected in Eppendorf tube. Extracted oil was diluted in hexane. Filtered after separation. This process was done separately for all three Ocimum species and kept in separate Eppendorf tube, and symbolized it as O.t, O.b and O.g

3.13.1 Separation of extracted oil by TLC method (Izmailov & Shraiver, 1938):

TLC stand for Thin Layer Chromatography. This technique is invented by Russian scientist N.A. Izmailov & M.S. Shraiber in 1938.Oil component is separated by chromatography method as below.

Extracted oil of selected Ocimum species has been loaded on the spot of Silica gel Plate for TLC. Now Plate was dip into solvent (Benzene + Methanol in the ratio of 10:1) & for Linalool and Geraniol whenever Toluene + Ethyl acetate + Acetic acid in the ratio of 8:2:0.5 for. Eugenol and Thymol, as running solvent.After run the solvent plate was removed and kept for dry the solvent.After dry Plate was incubated at 60^0C for ½ hours. After ½ plate was removed from incubator because spot has developed. R_f value was determined by using the following formula.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

3.14 Molecular Characterization

Molecular Characterization determines the genetic Characteristics of the organism in plant or animals. For molecular Characterization of three Ocimum species, following steps are involved.

3.14.1 Isolation of DNA

DNA first isolated by Firedrich Miescher in 1869. It is made up of three different types of compounds, Sugar molecules, Phosphoric acid & Nitrogenous bases. DNA isolation is the first step in the study of specific DNA sequences or molecular characterization.

Materials & Method:

- Preparation of DNA extraction buffer. Mixed the following in the appropriate quantity.

  1. Tris 2.24 gram
  2. EDTA 1.16 gram
  3. NaCl 16.352 gram
  4. 4gram CTAB
  5. 4 ml Marcaptoethanol (2%)

  6.4 gm PVP

  7. Made the volume 200ml with D.W.

  Adjusted pH 8.0 with help of 1N HCl

Steps of DNA isolation

500 mg each of *O. tenuiflorum* L., *O. gratissimum* L., and *O. basilicum* L. leaf was taken and kept in separate centrifuge tube. 700ml µl DNA Extraction buffer was added in each centrifuge tube.Stirred it with a glass rod for proper ejection of DNA molecules. Water bathed at 60^0C for 1 hour. Left it at room temp. for 20 minutes. Vortexes for one min. centrifuged it for 10 minutes at 700 RPM. Collected the supernatant of each centrifuge tube. Added the mixture of isoamyl alcohol and chloroform in a ratio of 24:1 and left it for 25 minutes.A white precipitate is formed like that fine thread or cotton, in each

tube.Again centrifuged for 10 minutes at 12000 RPM. Inverted the tube and washed it 3 times with 70% alcohol. Added 20µl T_{10} E_1 for proper dissolution of DNA molecules. Now the sample is ready for running in agarose gel.

3.14.2 Agarose Gel Electrophoresis (Arna Tiselius, 1930)

Agarose Gel Electrophoresis is a technique employed to separate DNA or RNA fragment based on their size, utilizing and electric field. Nucleic acid being negatively charged will migrate towards the positive pole or anode during agarose gel electrophoresis, as shown in below Figure.

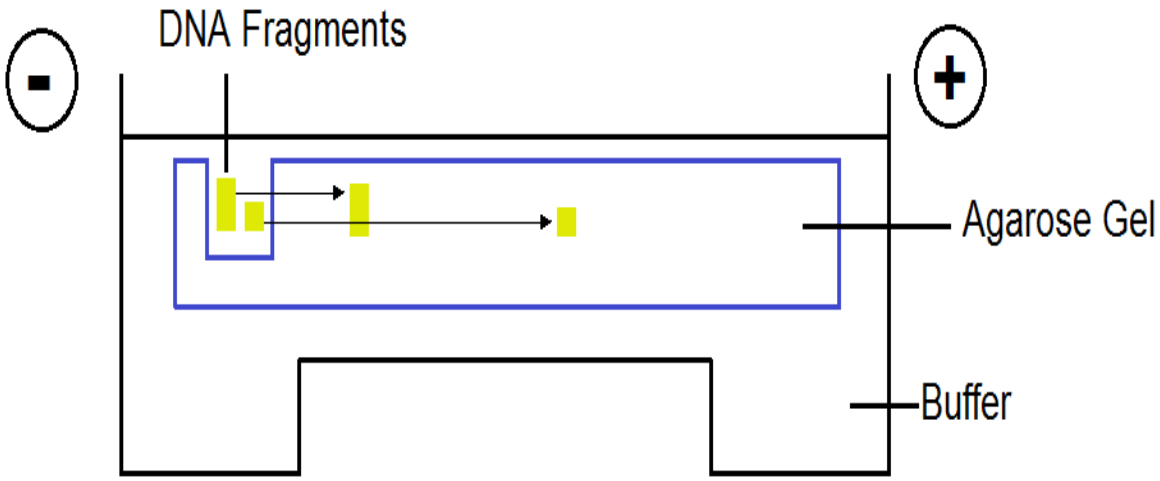


Figure 3.2: Migration of negatively charged DNA in the agarose gel

- Agarose, a high molecular weight polysaccharide extracted from the walls of marine algae.
- Agarose is insoluble in cold water but dissolves in boiling water.

Materials of Agarose gel electrophoresis:

Stock solution.

10 X TBE/50XTAE

Et. Br (10mg/ml)

Loading Buffer (6x)

Equipment:

Microwave oven	Gel casting plate
Horizontal Gel Electrophoresis system	Comb
UV-Transilluminator	Power Pack
Camera	

Method:

Dissolved 242 gram Tris base and 37.2g Na_2 EDTA ($2H_2O$) in 900 ml of D. D. Water. Added 57.1 ml glacial Acetic acid and adjusted the final volume to 1 litre. Diluted 2ml of 50X 7AE – buffer in 100ml D.D.W in a measuring flask. Now 2.0gram agarose powder weigh out and dissolved in 50 x TAE buffer and it was boiled in Microwave oven for 2 min. After heating EtBr was also added. After slightly hot to the hand of agarose solution, it was poured in gel casting tray and placed comb.

Loading the Gel

Gel was mounted in the electrophoresis tank and flood the top of the gel with fresh runing buffer 1X TAE to covered the gel to depth of about 1mm.DNA sample was loaded through micropipette.

Starting the Run

When all the three DNA samples have been loaded, closed the lid of the gel tank and attached the electric leads and turned on the power supply so that DNA migrated towards anode (red lead). Applied voltage, 50 Volts (40-60 volts). After few minutes, BPB began migrated from the well into the body of the gel.

3.14.3 DNA-Quantification

There are two widely used methods to quantitate DNA (Sambrook et al, 1989). One is the spectrophotometric measurement of the amount of UV-radiation absorbed on the basis of DNA and other in comparison of

fluorescent intensities of unknown and standard DNA sample by running agarose gel and then observing under UV-light. In this work, spectrophotometric method has been used.

Spectrophotometric method: - In this method following formula is used.

$$\text{DNA concentration (mg/µl)} = \frac{\text{O.D.260 x dilution factor x 50}}{1000}$$

3.14.4 Polymerase Chain Reaction, (Kary Mullis et al 1985)

The technique PCR is invented by Kary Mullis in 1985, in which a small amount of DNA can be amplified in large quantities in short time.

Requirements:

- PCR – Machine
- Micropipette
- Cocktail with its tray
- Micropipette tips
- Primer P_1& P_2
- dNTPs
- Taq Polymerase
- Buffer
- D. Water (DDW)

Procedure:

For PCR, three separate sterilized coacktail has been taken and symbolized it for *O. tenuiflorum, O. gratissimum*, and *O. basilicum* as, O.t. O.g and O.b through thin printed marker. All the three coacktail has been kept on tray and it was prepared for PCR Programme as below in table No. 3.1

Table 3.1: Preparation of Master Mix (For 50 μl) for PCR

Contents	O.t (μl)	O.g (μl)	O.b (μl)
DNA P1 P2 dNTPs (2.3MM) Buffln (10x) Taq Polymerase Water	1 2 2 4 10 1 30	1 2 2 4 10 1 30	1 2 2 4 10 1 30
Total Volume	**50**	**50**	**50**

After preparation of Master mix all the three coacktail carried for PCR Programme. Machine was started after setting programme. PCR was completed in following cycling conditions as in table no 3.2

Table 3.2:PCR-Cyclic Condition

S.N.	Cyclic conditions	Temp	Time	Cycles
1	Initial Denaturation	94^0C	3 min.	30 Cycles
2	Denaturation	94^0C	1 min.	
3	Annealing	50^0C	1 min.	
4	Extension	72^0C	2 min.	
5	Final Extension	72^0C	7 min.	

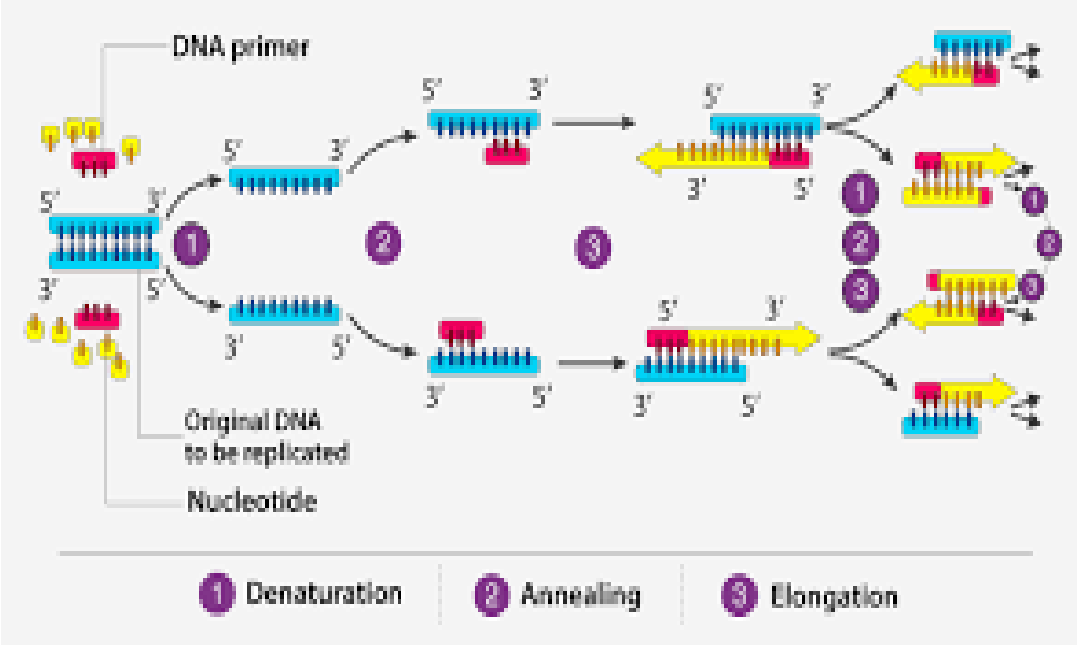


Figure 3.3: Representation of PCR

After completion of PCR Programme, the PCR products were checked on 2% agarose gel for the amplification. Before loading into the wells, gel loading dye was added to the sample. After loading the gel, the samples were run under constant voltage condition (80 volt) till the two dyes gel separated. Amplification products appeared as sharp orange colour bands under UV transilluminator due to intercalation of ethidium bromide, as in figure-4.18. There are 4 lanes in a gel, one for ladder and 3 lanes are Ocimum species, symbolized as L,1,2 & 3 (L-for Ladder) *O.tenuiflorum* L., *O.basilicum* L. & *O.gratissimum* L. respectively.

3.14.5 SANGER SEQUENCING: -It is DNA sequencing technique uses electrophoresis to determine the sequence of a small targeted regions of genome. It is also known as "Chain termination method". This method was developed by Fredrick Sanger and his colleagues in 1977. After his name, the name of this marvellous technique is Sanger Sequencing.

Materials: -

(a) Single Stranded DNA

(b) dNTP

(f) Coacktail

(g) Micropipette

(c) ddNTP

(d) Primer

(e) DNA Polymerase

(h) Micropipette tip with box

(i) Gel electrophoresis Appratus

(j) UV – Illuminator

Method: -

Four cocktails has been taken each for three Ocimum species. 4 micro litre denatured DNA has been taken in each cocktail through micropipette for Ocimum species. 2 micro litre dNTPs was also added. Now 2 micro litre ddNTP was added. 2 micro litre DNA polymerase was added and lastly 2 micro litre primer was added. It was cyclomixed, shown as in figure No- 3.4, 3.5 & 3.6.

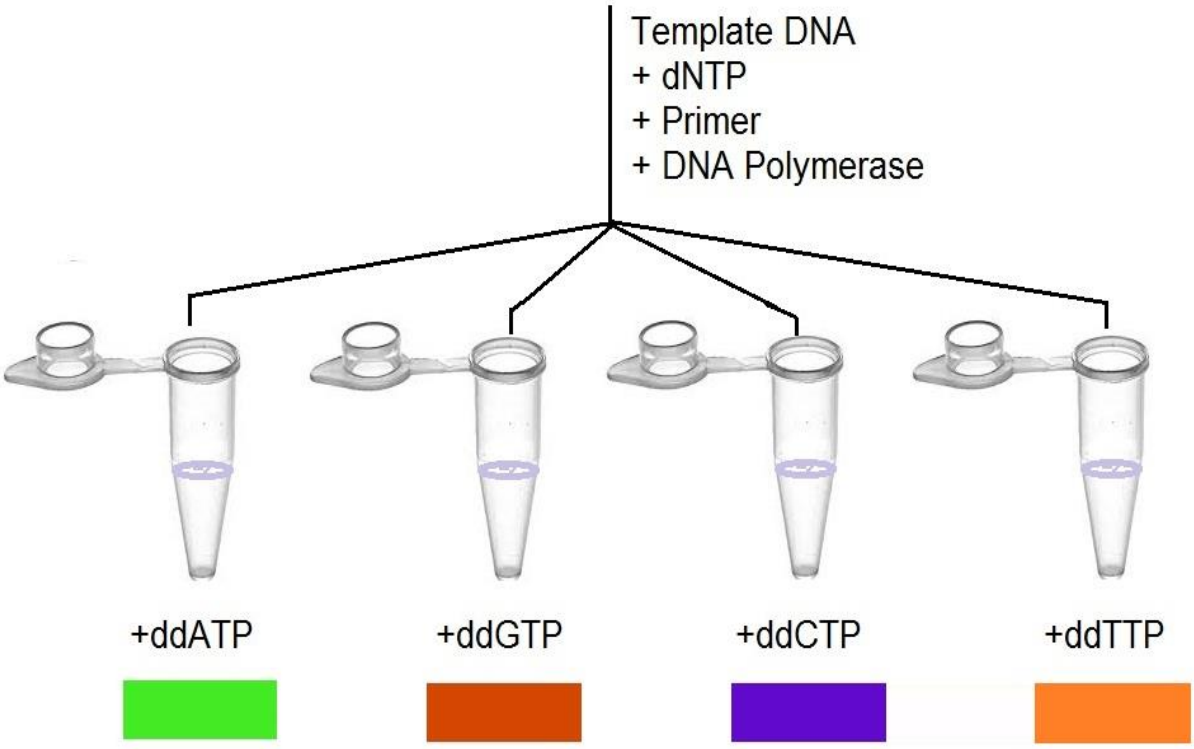


Figure 3.4: Prep. of mastermix of Sanger Sequencing for *Ocimum tenuiflorum* L.

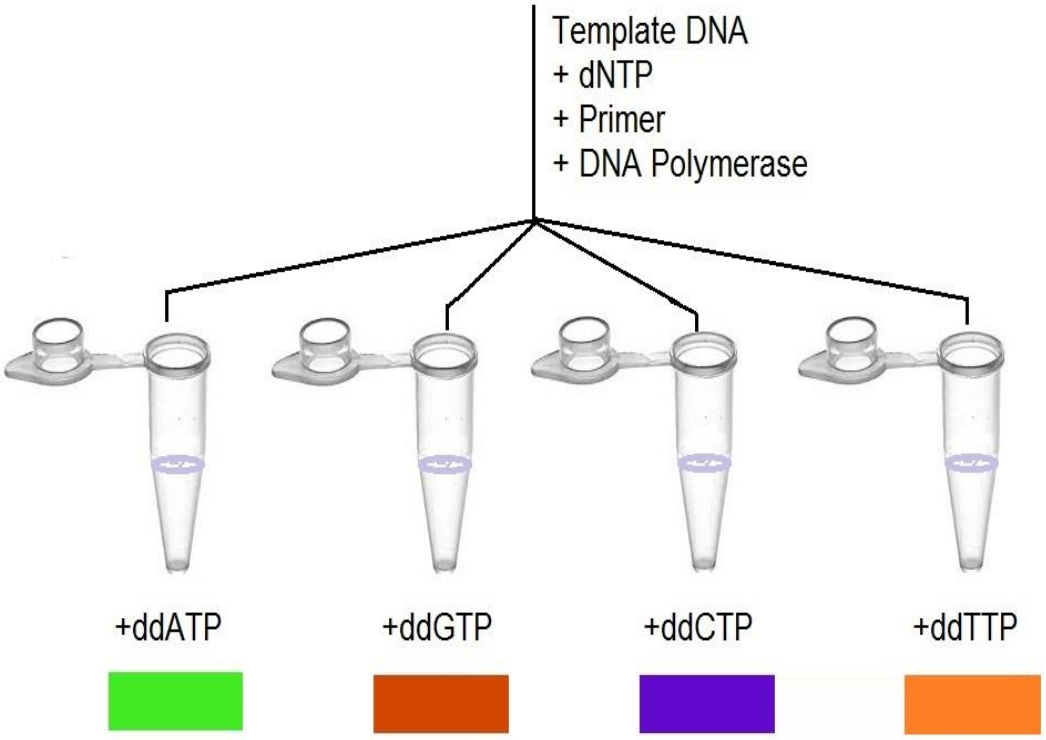


Figure 3.5: Prep. of mastermix of Sanger Sequencing for *Ocimum basilicum* L.

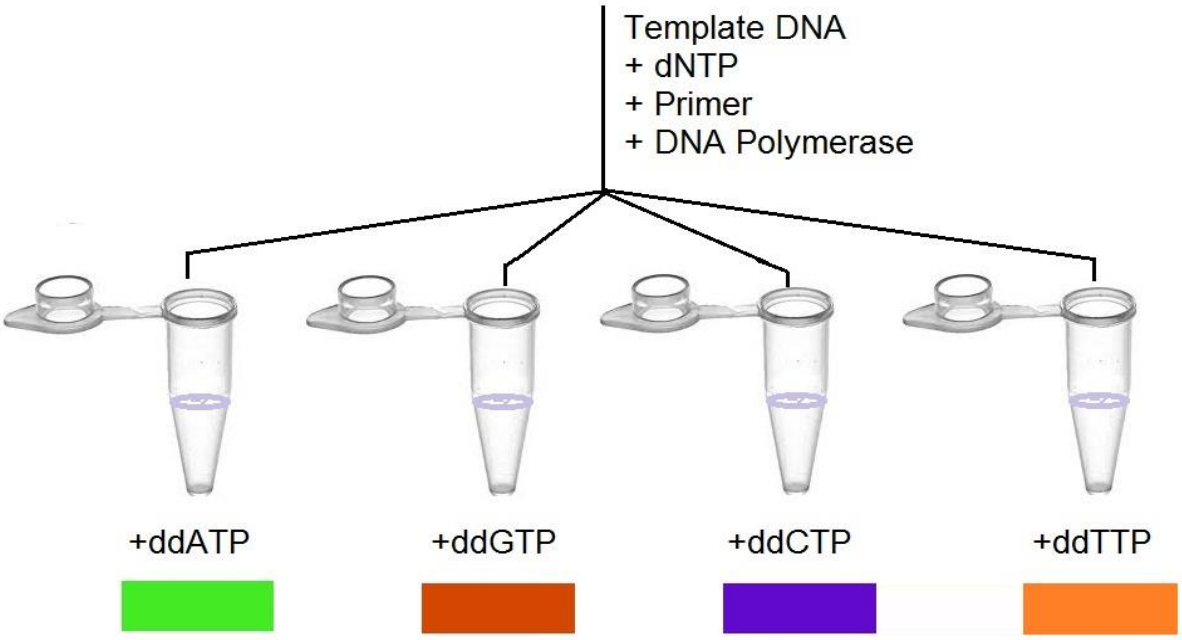


Figure 3.6: Prep. of mastermix of Sanger Sequencing for *Ocimum gratissmum* L.

After preparation of Mastermix the content of the tube was transferred to the capillary tube (containing gel) in the machine of Sanger sequencing, which is connected with Laser and computer screening. According to molecular weight, the bases of low molecular weight run fast and gave the position of peak through laser and then high molecular weight bases.

CHAPTER-4

RESULT & DISCUSSION

4. RESULT AND DISCUSSION

According to research topic "Phytochemical and Molecular Characterization in selected species of Ocimum in and around of Munger District," the whole work is divided into following five objectives.

4.1 Collection & identification of selected plant: Ocimum species has been collected in three places of Munger, out of ten visited area. These three places are Forest of Bheembandh, Fort area and area of KVK, as in table No-4.1 Soil of Bheembandh is reddish and yellow to greyish. Soil texture is loamy having pH 6.8. In this place, it was found that all the three species of Ocimum namely *O.tenuiflorum* L., *O.basilicum* L. & *O.gratissimum* L. have grown but number of *Ocimum basilicum* L. is more among four species,

Table 4.1: Texture, Color and pH of soil

Name of the Location	Soil Texture	Colour	pH
Bheem Bandh Area	Loamy	Reddish Yellow to Greyish	6.8
Fort Area	Sandy	Reddish – Grey	7.1
KVK Campus	Clay	Red	7.3

Loamy is a balance mixture of clay, silt and sand particle, this characteristic of soil is favorable for plant growing.

In the Red fort area of Munger District, the soil texture is sandy having color Reddish-Grey with pH 7.1. In this place only one types of species has found namely *O.tenuiflorum* L. Where in the area of KVK campus, the soil texture is clay having color Red with pH 7.3. In this area *O.gratissimum* L. is more than *O.tenuiflorum* L. Soil with higher clay content generally exhibit a higher buffering capacity than soil with little clay. Soil with high organic matter will have a higher sandy soil, tend to have a lower pH than clay soil. All the three plant were identified with the help of plant height, leaf size and leaf color as

in table no.4.2 Leaf color of *O.tenuiflorum* L. is purple having leaf size 2-6 cm long and 1-2.5 cm wide. Leaf color of *O.basilicum* is bright green having leaf 3-11 cm long and 1-6 cm wide, whenever leaf color is dark green having leaf size 2-5 cm long and 1-2 cm wide, details as in table No. 4.2, also justified by Dr. S. Hassan (2021), former HOD of PG Botany R.D.& D.J College, Munger.

Table 4.2: Identification of Ocimum species

Selected Species of Ocimum	Plant Height (m)	Leaf Size	Leaf Colour	Commonly known as
1. *O. tenuiflorum* L.	30-60 cm	2-6cm long 1-2.5cm wide	Purple	Krishna Tulsi (or Shyama Tulsi)
2. *O. Basilicum* L.	45-60 cm	3-11 cm long 1-6cm wide	Bright Green	Sweet Basil
3. *O. gratissimum* L.	60-120 cm	2-5cm long 1-2cm wide	Dark green	African basil or Van Tulsi

- After identification of Ocimum species photo was arrested in camera which are shown as in figure No. 4.1

Figure 4.1: Selected species of Ocimum.

3.2 Phytochemical Screening in selected plant species: Phytochemical characterization has been done by both qualitative and quantitative analysis. In qualitative analysis Ocimum extract reacts with biuret reagent, the colour turned violet-red indicates the present of protein. For confirmation, Ocimum extract was again heated with Millon's reagent, the colour turned pink, indicate the presence of protein.it was revealed that there are Protein, Sugar, Alkaloid, Flavonoid, Glycosides Terpenoid, Phenol and Steroid has been analyzed and these metabolites are present in the leaf extract of all the three selected species of Ocimum, which is also supported by Borah R & Biswass, P (2018). Observations and results are tabulated as in table no. 4.3

Table 4.3: Observation of primary & secondary metabolites

Test	Experiment	Observation	Result
Protein Test	[1] 2 ml Biuret reagent + 2ml extract in a test tube + mixed and Heated	It turned violet – red	May be protein
	[2] 2ml extract + 4ml Millon's reagent in a test tube + mixed heated	It turned pink colour	Protein confirmed
Carbo-hydrate Test	[1] 1ml Fehling sol"A + 1ml Fehling Sol"B + 2ml extract in test tube + mixed and heated	It turned red colour	May be sugar
	[2] 2ml Benedict reagent + 2ml extract in a test tube + mixed and heated	It turned brick red colour	Sugar confirmed
Alkaloid Test	[1] 2ml extract + 2ml of 1% Hcl in a test tube + Heat + Mayer's reagent	It turned white	May be alkaloid

	[2] 2ml extract + 2ml of 1% Hcl in a test tube + Heat + Wagner's reagent	It also turned white	alkaloid confirmed
Flavonoid Test	[1] 2ml extract + Little amount of Magnesium and conc Hcl dropwise [2] 0.5ml extract + 2ml of 2% NaOH Sol"	After few minutes it turned pink colour It turned yellow colour and become colourless after addition of dil. Hcl in Little amount	May be flavonoid Flavanoid confirmed
Glycoside Test	[1] 2ml extract + 2ml chlorofom + 2ml conc. H_2SO_4 in a test tube + shaked gently [2] 2ml extract + 2ml chloroform + 2ml acetic acid in a test tube + cooled + conc. H_2SO_4 in few drop	Reddish brown colour was appeared Colour changed from violet to greenish blue	May be glycosides Glycosides confirmed
Terpenoid Test	2ml extract + 2ml chloform a test tube + warmed till dryness + 2ml conc. H_2SO_4 + Heated 3 mins.	It turned grey colour	Terpenoid Present
Phenol Test	2ml alcohol + 2 drops Ferric chloride + 1ml of extract	It turned black colour	Phenol Present

Steroid Test	2 ml Plant Extract + 2 ml Chloroform + Cyclomixed + 2 ml conc H_2SO_4	It turned reddish brown color	Steroid Present

3.2.1 Quantitative analysis of protein: In quantitative analysis it was found that *O. tenuiflorum* L. has highest content of protein concentration out of three selected species of Tulsi. Supported by Sandeip Vidhani and et al 2006.Observations are in table No-4.4

Table 4.4: %T and O.D. of Bovine albumin

T.T. No.	Protein Conc. (µg)	Volume of working Solu. (ml)	Volume of D.W. (ml)	Volume of CuSO4 reagent (ml)	Wait for reaction (min.)	Volume of F.C reagent (ml)	%T against B	
							T%	O.D.
B.	0.0	0.0	1.0	5	10	0.5	93-100	
1	10	0.1	0.9	5	10	0.5	93	0.031
2	20	0.2	0.8	5	10	0.5	88	0.055
3	30	0.3	0.7	5	10	0.5	82	0.086
4	40	0.4	0.6	5	10	0.5	75	0.125
5	50	0.5	0.5	5	10	0.5	71	0.148
6	60	0.6	0.4	5	10	0.5	66	0.180
7	70	0.7	0.3	5	10	0.5	64	0.193
8	80	0.8	0.2	5	10	0.5	57	0.244
9	90	0.9	0.1	5	10	0.5	55	0.260
10	100	1.0	0.0	5	10	0.5	51	0.292

Total Volume = 6.5ml

The result has been determined after preparation of standard curve by Lowry method as below in figure No- 4.2.This is the most commonly used method for determining protein due to its sensitivity. When F.C reagent (a mixture of sodium phosphomolybdate and tungstate) react with $CuSO_4$ solution in alkaline medium, the peptide bond (-CO-NH) gives a blue colored complex which constitute the sensitivity. It is based on Biuret method, i.e. Peptide bond of protein reacts with Cu ion. This Cu-ion reacts with F.C reagent that for the reduction of phosphomolybdotungustate to keto-phosphomolybdenum blue by copper, catalyzed oxidation of aromatic acids (like tyrosine and tryptophan)

In this experiment, the relationship between protein concentration and the optical density（O.D.) was examined using the Bovine Albumin protein standard. The results obtained are summarized in Table No-4.4. The table shows data for percent transmittance (%T),O.D., and protein concentration from 0 μg to 100 μg. In this assay,the protein concentration was varied, and the corresponding light absorbance was measured, providing insights into how the presence of protein affects the light transmission and absorbance. The data in Table No-4.4 show a clear trend of decreasing percent transmittance (%T) asthe protein concentration increases. Conversely, the optical density (O.D.) increases with higher protein concentrations. At the lowest protein concentration (0 μg), the percent transmittance was between 93-100%, indicating almost no absorption of light by the sample.As the protein concentration increased, the %T decreased, while O.D.increased in a linear fashion, as detailed in the table.

Interpretation of Data from Table No-4.4

- **%T vs. Protein Concentration:**

The data show that at O μg of protein (entry B in Table No-4.4), the percent transmittance was high at 93-100%o.This indicates very little light absorption, as expected in the absence of protein. As the protein concentration increased, the percent transmittance steadily decreased. For

example, at 10 µg of protcin (entry1), the %T dropped to 93%, and at 100 µg of protein (entry 10), it decreased further to 51%. This inverse relationship between protein concentration and %T is typical of protein assays, as higher protein concentrations lead to greater light absorption, resulting in reduced transmittance.

- **O.D. vs Protein Concentration:**

In Table No-4.4, the optical density (O.D.) increased with increasing protein concentration. At 10 µg of protein (entry 1), the O.D.was 0.031, and at 100 µg（entry 10), it rose to 0.292. This trend aligns with the Beer-Lambert Law, where absorbance (O.D.) is directly proportional to the concentration of the absorbing species-in this case, the protein. The gradual increase in O.D. from 0.031 to 0.292 reflects the expected behavior for a protein assay, where the formation of the protein-dye complex enhances light absorption as the protein concentration rises.

- **Consistency and Sensitivity:**

The results in Table No-4.4 show consistent changes in %T and O.D. with increasing protcin concentration. This consistency suggests that the assay is reliable within the range tested (10 µg to 100 µg). The assay is also sensitive to small changes in protein concentration, as evidenced by the measurable changes in both %T and O.D.with incremental increases in protein concentration.

- **Potential Saturation Point:**

At the higher end of the protein concentration range (90 µg and 100 µg), the %Treaches 55% and 51%, respectively, while the O.D. reaches 0.260 and 0.292. This suggests that the assay may begin to approach saturation at these higher concentrations. It would be valuable to test even higher protein concentratins to determine the absorbance percentage, indicating a limit to the assay abilty to accurately mcasure protein concentration at higher levels.

- **Control and Reagent Volume Consistency:**

  The volume of the $CuSO_4$, reagent and F.C. reagent remained constant a 5 ml and0.5 ml, respectively, across all samples, as noted in Table No-4.4. This controlled experimental setup ensures that variations in %T and O.D. are primarily due to changes in protein concentration rather than reagent volume.The data presented in Table No-4.4 demonstrate the expected relationship between protein concentration and absorbance in a protein assay. The decrease in %T and corresponding increase in O.D. as protein concentration rises confirms the sensitivity to changes in protein concentration. The assay appears to be accurate and reliable within the tested concentration range, and there is evidence that it may reach a saturation point at very high protein concentrations.

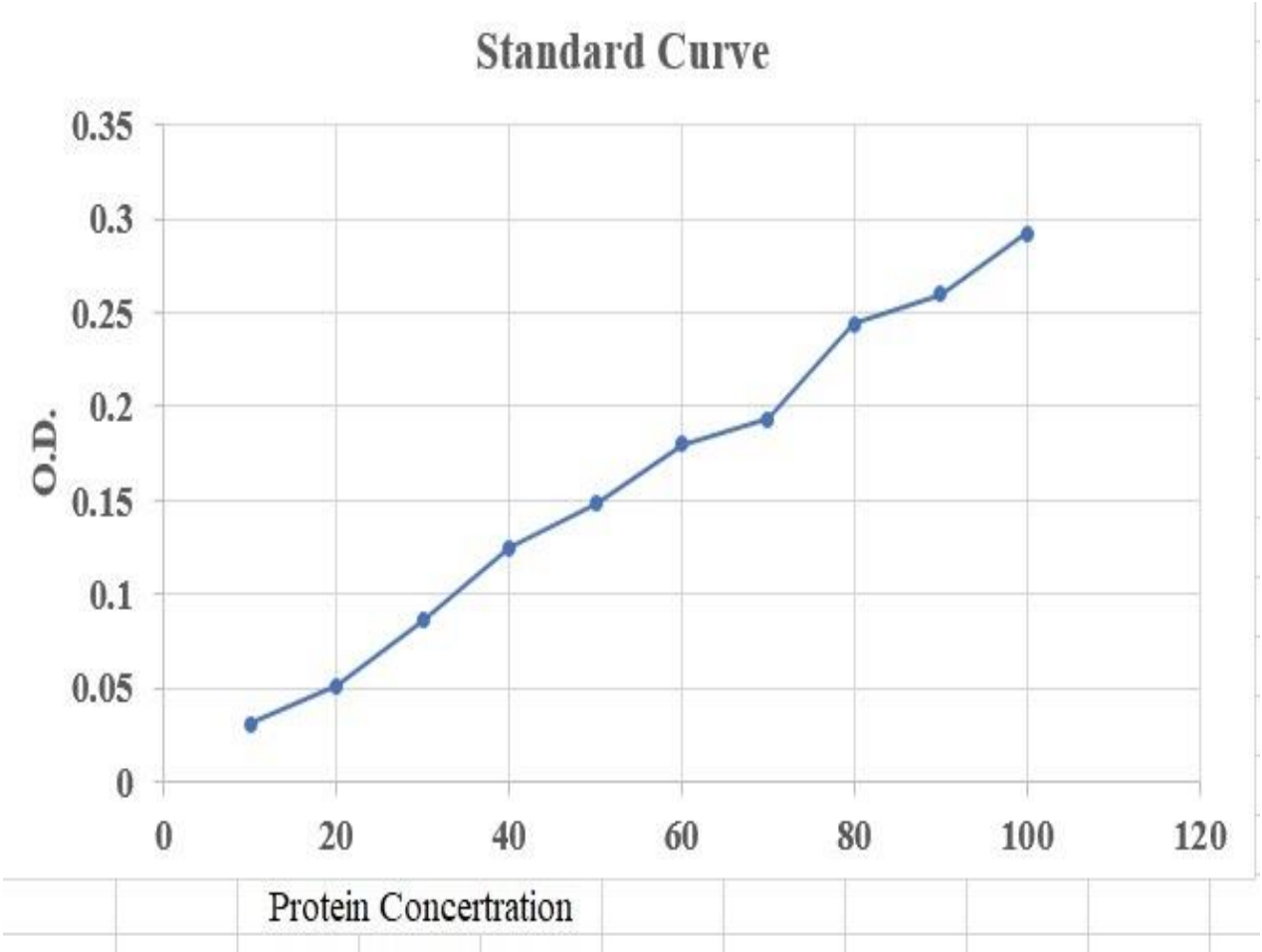


Figure 4.2: Standard curve of Protein by Lowry Method

Future work could involve exploring even higher concentrations to fully characterize the upper limits of the assay sensitivity and accuracy. Overall, the results validate the use of this assay for protein quantification in the given range.

- **Observation of protein concentration in three species of Ocimum using standard curve.**

Control T.T.	T% 86→100

Table No. 4.5: Optical density of Ocimum Species

Test tubes No.	Plant Species	Optical Density (O.D.)
1	*O. tenuiflorum* L.	0.13
2	*O. basilicum* L.	0.11
3	*O. gratissimum* L.	0.12

From the above table no.- 4.5 The value of O.D has been compared and coincide from standard curve of protein as followed by Figure-4.2, the concentration value has been determined as in table no-4.6.

4.2.2 Quantitative analysis of sugar

When a range of ion concentration of glucose solution is treated with alkaline copper reagent, it reduces copper and the glucose is oxidized. The reduced copper reacts with arsenomolybate and produce molybdenum blue, a violet colored complex of different absorbance. This absorbance can be measured with the help of colorimeter at 540 nm as in table No-4.7

Table 4.6: Protein Concentration of Ocimum Species

Test tubes No.	Plant Species	(O.D.)	Protein concentration µg/ml
1	*O. tenuiflorum* L.	0.13	40
2	*O. basilicum* L.	0.11	36
3	*O. gratissimum* L.	0.12	38

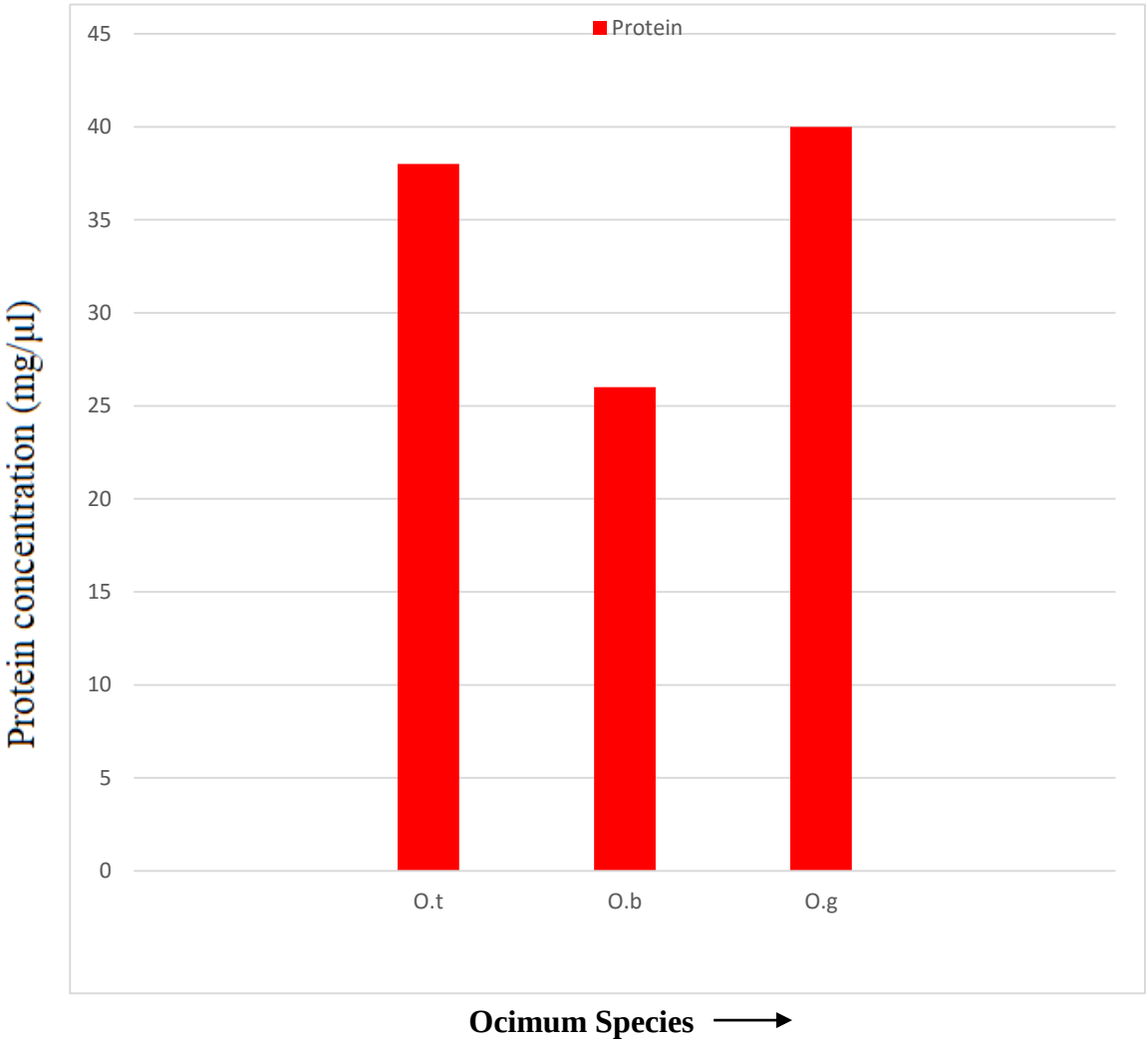


Figure 4.3: Bar Graph showing protein concentration

Percentage transmittance (T %) and corresponding optical density (O.D) is helpful in calculating the reducing sugar.

Table 4.7: % T & O.D. of glucose solution

T.T. No.	Glucose Conc. (ug)	Vol. of W.S (ml)	Vol. of D.W (ml)	Vol. of Nelson's reagent (ml)	Arseno molybdate reagent (ml)	Vol. of D.W	% T against B	
							T%	O.D.
B.	00	0.0	1.0	1.0	1.0	7.0	96-100	
1	10	0.1	0.9	1.0	1.0	7.0	90	0.045
2	20	0.2	0.8	1.0	1.0	7.0	80	0.096
3	30	0.3	0.7	1.0	1.0	7.0	73	0.136
4	40	0.4	0.6	1.0	1.0	7.0	65	0.187
5	50	0.5	0.5	1.0	1.0	7.0	58	0.236
6	60	0.6	0.4	1.0	1.0	7.0	52	0.284
7	70	0.7	0.3	1.0	1.0	7.0	49	0.310
8	80	0.8	0.2	1.0	1.0	7.0	45	0.347
9	90	0.9	0.1	1.0	1.0	7.0	40	0.398
10	100	1.0	0.0	1.0	1.0	7.0	35	0.456

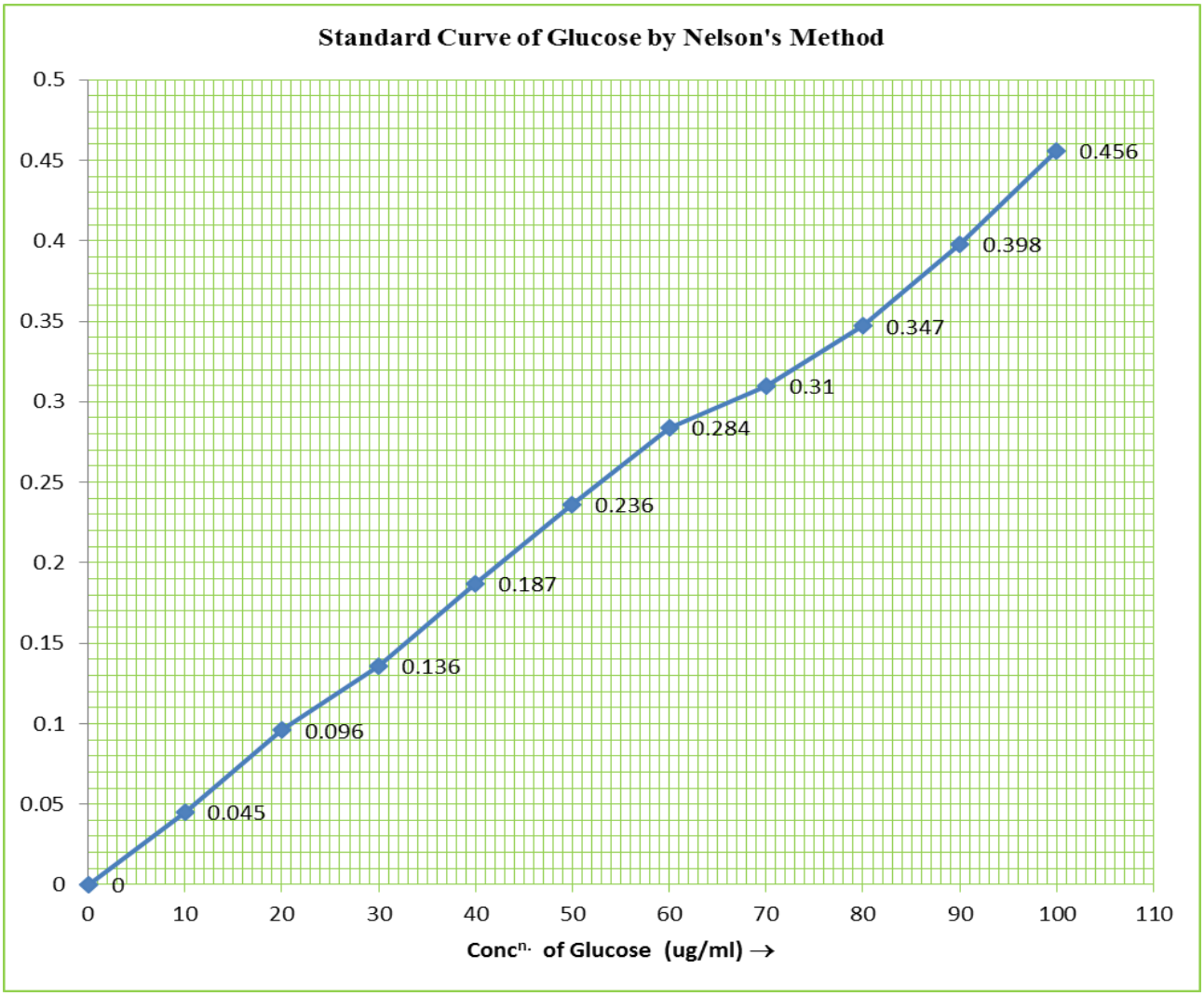


Figure 4.4: Standard curve of glucose by Nelson's Method

- **Observation for optical density of sample for sugar**

T.T. No. – 5 Control	T% 92→100

Table 4.8: Optical density of Ocimum species for Sugar

Test tubes No.	Plant Species	Optical Density (O.D.)
1	*O. tenuiflorum* L.	0.038
2	*O. basilicum* L.	0.044
3	*O. gratissimum* L.	0.040

Optical Density (O.D) of the plant species has been Coincide with standard curve of Glucose as shown in Figure No-4.4 and amount of sugar in mg/100mg of three selected species of ocimum has been determined with the help of factor as shown in table No-4.9

Factor: x μg reducing sugar = 0.1 ml supernetant

→ 10x μg reducing sugar = 1.0 ml supernetant

→ 30x μg reducing sugar = 3.0 ml supernatant

→ 30x μg reducing sugar = 0.1 ml homogenate

→ 300x μg reducing sugar = 1.0 ml homogenate

→ 300x/1000 μg reducing sugar = 100 mg Tulsi Leaf

→ f = 0.3

Table 4.9: Table showing amount of sugar present in Ocimum Species

T.T. No.	Plant Species	Factor (f)	Concentration in μg (x)	Amount (mg/100mg) (f x)
2	*O. tenuiflorum* L.	0.3	8.6	2.58
3	*O. basilicum* L.	0.3	9.6	2.88
4	*O. gratissimum* L.	0.3	9.2	2.76

Table 4.9 presents the quantitative estimation of sugar content in three species of Ocimum like *O. tenuiflorum* L., *O. basilicum* L., *and O. gratissimum* L. expressed as milligrams of sugar per 100 mg of fresh plant material. The determination was conducted using a colorimetric method, where the optical density of the test samples was compared with a glucose standard, and a specific conversion factor (f) was applied to determine the actual concentration of sugars. As seen in Table 4.9**,** all three Ocimum species exhibit measurable amounts of sugar, with the values ranging from 2.58 mg/100 mg to 2.88 mg/100 mg of fresh weight. Each test tube (T.T. No. 1 to 4) was associated with a specific plant species, and the concentration in

micrograms (x) derived from the standard curve was multiplied by a uniform factor (f = 0.3) to calculate the final sugar content.

Key Finding

- *O. basilicum* L. shows the highest sugar content, with a calculated value of 2.88 mg/100 mg, corresponding to an optical concentration of 9.6 µg.

- *O. gratissimum* L. records a slightly lower sugar concentration of 2.76 mg/100 mg, based on a value of 9.2 µg.

- *O. tenuiflorum* L. demonstrates the lowest sugar content, with 2.58 mg/100 mg, derived from 8.6 µg concentrations.

These variations, although subtle, may reflect interspecific differences in primary metabolism, photosynthetic capacity, or developmental stage at the time of sampling. The slightly higher sugar content in *O. basilicum* L. could be indicative of their relatively more active carbohydrate metabolism or a greater accumulation of soluble sugars, which may contribute to their characteristic aromatic and medicinal properties. On the other hand, *O. tenuiflorum* L. lower sugar content might be linked to species-specific traits or environmental conditions during collection. The use of a constant factor (f = 0.3) across all samples ensures standardization, allowing for accurate comparative analysis between species. The calculated values represent a reliable index of sugar concentration per 100 mg of plant material, a useful metric for biochemical profiling and nutraceutical evaluation.

In summary, Table 4.9 highlights that sugar content among different Ocimum species varies slightly but significantly, with *O. basilicum* L. exhibiting comparatively higher levels. This biochemical trait may contribute to their broader use in traditional medicine and culinary applications, as sugars are not only nutritional components but also influence flavor and plant stress responses.

From above observation it was found that *O. tenuiflorum* L. (Shyama Tulsi) has lowest amount of sugar among three selected species of ocimum. It is also supported by Simarnjeet Kaur et al. 2020**.** This result was analyzed by Nelson's Method through standard curve, when a range of concentrations of Glucose.

4.2.3 Quantitative analysis of alkaloid

Similarly Analysis of secondary metabolities likes alkaloid, Flavonoids, Glycoside,Terpenoid and Phenol, it was found that the percentage amount of alkaloid present in *O. tenuiflorum* L., *O. basilicum* L. and *O. gratissimum* L. are 1.18, 0.96 & 1.10 respectively for table No-4.10, 4.11 & 4.12 as in table No-4.15. It was found that has highest conduct of alkaloids supported by Borah R & Biswas S.P, 2018, who observed the amount of alkaloid ranged from 0.91% to 1.28%; Alkaloids are a group of naturally Occurring chemical compounds that have a wide range of health benefit they are known to have antioxidant, anti-inflammatory and anti-cancerous properties.

Table 4.10: Amount of alkaloid in *O.tenuiflorum* L.

No. of Obs.	Ocimum Species	Initial wt. W1 (gms)	Final wt. W2(gms)	Difference (W2-W1)	Average (gms)
1	*O. tenuiflorum* L.	0.050	0.110	0.060	0.059
2	*O. tenuiflorum* L.	0.050	0.109	0.059	
3	*O. tenuiflorum* L.	0.050	0.108	0.058	

$$\% \text{ Alkaloids} = \frac{(W2-W1)}{Wt.of sample} x\ 100$$

$$= \frac{0.059}{5} x\ 100$$

$$= 1.18\%$$

The present observation focuses on determining the alkaloid contentin *Ocimum tenuiflorum* by analyzing the weight difference before and after the

extraction or experimental treatment. All three observations were performed with a consistentinitial weight (W1) of 0.050 gms. The final weights (W2) post-treatment rangedfrom 0.108 to 0.110 gms, indicating an increase in weight, which may beat tributed to the detection or binding of alkaloid compounds.

The calculated weight differences (W2- W1) for the three samples are 0.060gms, 0.059 gms, and 0.058 gms, respectively. The average difference across the observations is 0.059 gms, reflecting a uniform and reproducible pattern ofalkaloid presence in *O. tenuiflorum* samples. This consistent weight increase suggests the presence of alkaloids in measurable and nearly similar quantities across all three replicates, highlighting the species phytochemical stability under standardized testing conditions. The Presence of alkaloids contributes significantly to its defensive, physiological and medicinal attributes.

Key Findings:

- **Uniform Sampling:** Each *O. tenuiflorum* sample was taken with a standardizedinitial weight of 0.050 gms, cnsuring comparability across observations.
- **Detectable Alkaloid Presence:** The increase in final weight indicates the successful detection or quantification of alkaloids in the samples.
- **Minimal Variation in Results:** The differences in weight (0.058-0.060 gms) are closely clustered, suggesting high reproducibiity and low experimental error.
- **Average Alkaloid Content:** The mean weight difference of 0.059 gms represents the average alkaloid content retained or detected in the samples under the given conditions.
- **Phytochemical Consistency:** The uniformity in alkaloid content across replicates reflects the phytochemical consistency of *Ocimum tenuiflorum*, which is crucialfor its application in traditional and modern phytotherapy.

Table 4.11: Amount of alkaloid in *O.basilicum*

No. of Obs.	Ocimum Species	Initial wt. W1 (gms)	Final wt. W2(gms)	Difference (W2-W1)	Average (gms)
1	*O. basilicum* L.	0.050	0.098	0.048	0.048
2	*O. basilicum* L.	0.050	0.100	0.050	
3	*O. basilicum* L.	0.050	0.098	0.048	

$$\% \text{ Alkaloids} = \frac{(w2-W1)}{Wt.of sample} x\ 100$$

$$= \frac{0.048}{5} x\ 100$$

$$= 0.96$$

The experiment aimed to estimate the alkaloid content in *Ocimum basilicum* L. commonly known as Sweet Basil by measuring the weight change before and after the alkaloid extraction or detection process. All three observations used aconstant initial weight (W1) of 0.050 gms for standardization. The final weights (W2) after processing ranged from 0.098 to 0.100 gms, resulting in weight differences of 0.048 gms, 0.050 gms, and 0.048 gms.The average weight difference, representing the estimated alkaloid content, was calculated as 0.048 gms.

This modest but consistent increase in weight across all replicates indicates thepresence of alkaloids in *O. basilicum* L. though in slightly lower quantity compared to other Ocimum species such as *O. tenuiflorum* L. as per related tables.

Key Findings:

- **Standardized Sample Weight:** Each sample of *O. basilicum* L. was taken with a fixed initial weight of 0.050 gms, ensuring consistent baseline conditions.

- **Consistent Alkaloid Detection:** The final weight differences (ranging from 0.048to 0.050 gms) confirm the presence of alkaloids in all samples, with minimal variation.

- **Average Alkaloid Yield:** The mean weight difference of 0.048 gms indicates amoderate alkaloid content in *O. basilicum* L. suggesting it has comparatively lower alkaloid concentration than *O. tenuiflorum* L. under similar experimental conditions.

- **High Reproducibility:** The narrow range of observed differences reflectsa high degree of reproducibility and reliability in the extraction or measurement method used.

- **Implication for Phytochemical Research:** The data positions *O.basilicum* L. as a stable, though relatively lower, source of alkaoids among the tested Ocimum species, which may influence its applications in medicinal and nutraceutical formulations.

Table 4.12 : Amount of alkaloid in *O.gratissimum* L.

No. of Obs.	Ocimum Species	Initial wt. W1 (gms)	Final wt. W2(gms)	Difference (W2-W1)	Average (gms)
1	*O. gratissimum* L.	0.050	0.107	0.057	0.055
2	*O. gratissimum* L.	0.050	0.105	0.055	
3	*O. gratissimum* L.	0.050	0.104	0.054	

$$\% \text{ Alkaloids} = \frac{(W2-W1)}{Wt.of sample} x\ 100$$

$$= \frac{0.055}{5} x\ 100$$

$$= 1.10\%$$

The present analysis was conducted to quantify the alkaloid content in *Ocimum gratissimum* through weight measurements taken before and after the alkaloid extraction or detection process. Each sample had a uniform initial weight (W1) of 0.050 gms**,** which served as the baseline.

The final weights (W2) for the three replicates were 0.107 gms, 0.105 gms, and 0.104 gms, respectively.The corresponding weight differences(W2-W1),indicating the presence of alkaloid content, were 0.057 gms, 0.055 gms, and 0.054 gms.The average weight difference was computed to be 0.055 gms.This consistent increase in weight reflects a reliable detection of alkaloid compounds in *O. gratissimum* L. The associated alkaloid percentage was calculated as 0.97%**,** indicating a moderately high alkaloid yield compared to other Ocimum species evaluated in related tables.

Key Findings:

- **Standardized Procedure:** All three samples of *O. gratissimum* L. were measured with a consistent initial weight of 0.050 gms, ensuing accuracy in comparison.
- **Reliable Alkaloid Detection:** The observed weight gains (0.054-0.057 gms) confirm the presence of alkaloids in all replicates, with minimal deviation, indicating reproducibility.
- **Average Alkaloid Yield:** The mean difference of 0.055 gms suggests that *O.gratissimum* L. contains a notable quantity of alkaloids.
- **Alkaloid Concentration:** The alkaloid content was determined to be 0.97%, placing *O. gratissimum* L. among the higher-yielding species in terms of alkaloid presence.
- **Pharmacological Relevance:** Given its relatively high alkaloid content, *Ocimum gratissimum* L. may be considered a promising candidate for further phytopharmacological studies and potential therapeutic applications.

  From Table 4.10, 4.11 & 4.12 we can write the % amount of alkaloids as shown below in table No- 4.13

Table 4.13: Comparative amount of % alkaloid

S. No.	Plant Species	% alkaloids
1	*O. tenuiflorum* L.	1.18
2	*O. basilicum* L.	0.96
3	*O. gratissimum* L.	1.10

The data presented in Table 4.13 summarizes the percentage of alkaloids detected across three Ocimum species: *O. tenuiflorum* L., *O.basilicum* L., and *O. gratissimum* L. The findings indicate variation in alkaloid concentration among species, reflecting the influence of genetic, biochemical, or ecological factors affecting secondary metabolite production.

- *O. tenuiflorum* L. showed the highest alkaloid content at 1.18%,
- *O. gratissimum* L. exhibited a moderate level of alkaloids (1.10%).
- *O. basilicum* L. displayed the lowest alkaloid concentration at 0.97%.

This variation suggests that different Ocimum specics have distinct phytochemical profiles, which may affect their medicinal and therapeutic applications, particularly those related to alkaloid-associated biological activities such as antimicrobial, analgesic, or anti-inflammatory effects.

Key Findings:

- **Highest Alkaloid Content:** *O. tenuiflorum* L. cxhibited the highest alkaloid percentage（1.18%), indicating its strong potential for medicinal use where alkaloids are the active constituents.

- **Close Similarity in Two Species:** *O. tenuiflorum* L. (1.18%) and *O. gratissimum* L.（1.10%) had closely comparable alkaloid levels, suggesting possible phytochemical overlap or taxonomic proximity.
- **Moderate Yield in *O. gratissimum* L.*:*** With 1.10% alkaloids, *O. gratissimum* L. also presents a promising profile for pharmacological applications.
- **Lowest Yield in *O. basilicum* L.*:*** *O. basilicum* L. had the lowest alkalid content（0.96%), which may influence its preference for culinary over medicinal use, though still biologically significant.
- **Species-Based Variation:** The variation in alkaloid concentration emphasizes the importance of species selection when developing herbal formulations or conducting pharmacognostic evaluations.

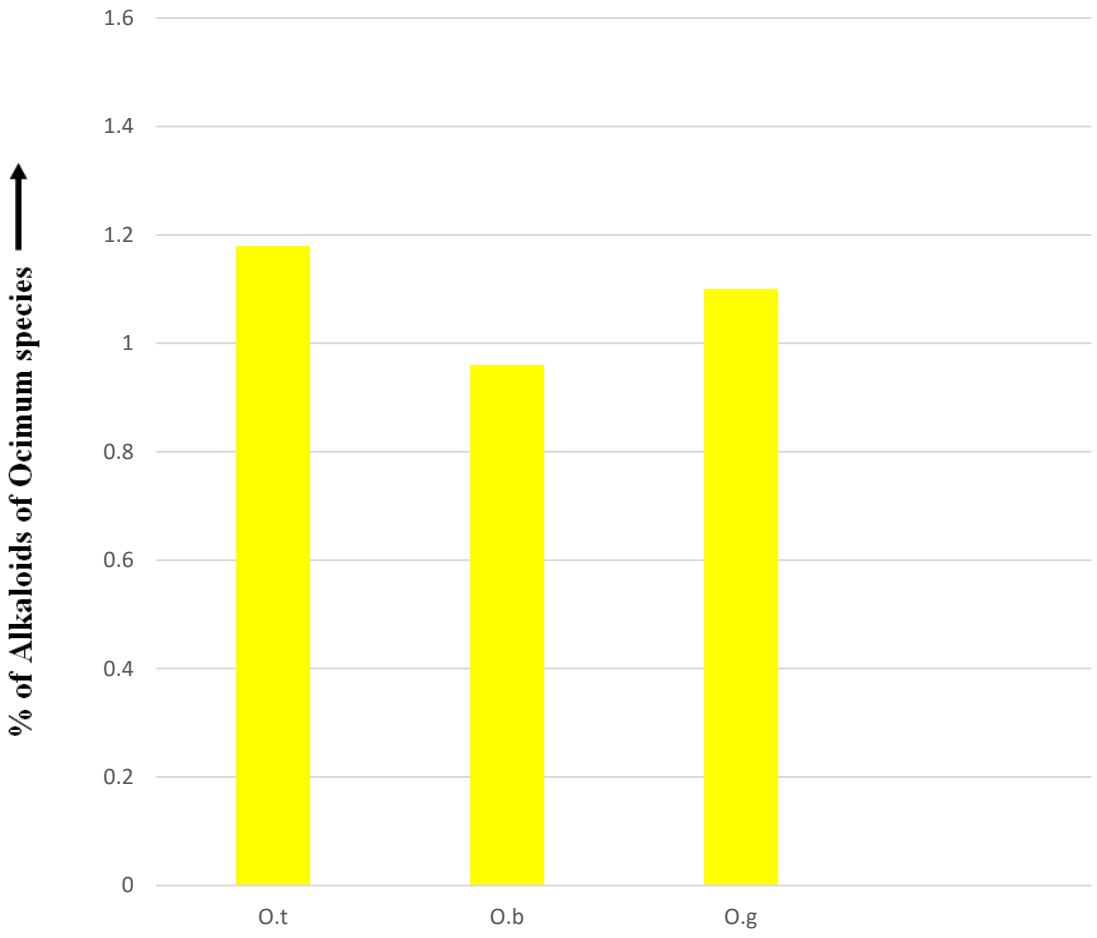


Figure 4.5: Bar Graph showing Alkaloid concentration

From above observation it was found that *O. tenuiflroum* L. has highest % amount of alkaloids among three selected species of Ocimum. It was also shown in Bar graph as in figure No- 4.5.They can also help to reduce cholesterol levels, improve digestion and boost the immune system. It is also supported by A.N.M. Mamun-or-Rashid at et al 2013, Borah R & Biswas S.P., 2018 and Md. Abdullah Jahanger &Asha Mishra 2023.

4.2.4 Quantitative analysis of Flavonoids: Flavonoids are analyzed by $AlCl_3$ method, through standard curve with the different concentration of Quarcetin and get richest amount of flavonoid in *Ocimum tenuiflorum* L. （Shyama Tulsi) among three selected species of Ocimum as shown in table no 4.14.

Table 4.14: O.D. of quarcetin in different concentration

T.T. No.	No. of quarcetin (ml)	Vol. of methanol (ml)	Concentration of quarectin	Vol. of 10% Alcl3 (ml)		Vol. of Sod. acetate		O.D.
B.	0.0	1.0	00	2ml		2ml		
1	0.1	0.9	10	2ml		2ml		0.12
2	0.2	0.8	20	2ml		2ml		0.23
3	0.3	0.7	30	2ml	Cyclomixed	2ml	Cyclomixed	0.32
4	0.4	0.6	40	2ml		2ml		0.41
5	0.5	0.5	50	2ml		2ml		0.53
6	0.6	0.4	60	2ml		2ml		0.62

7	0.7	0.3	70	2ml		2ml		0.73
8	0.8	0.2	80	2ml		2ml		0.82
9	0.9	0.1	90	2ml		2ml		0.92
10	1.0	0.0	100	2ml		2ml		1.12

Standard curve of flavonoid: -A standard curve has been drawn in which Concentration of quarcetin (µg/ml) has plotted on x-axis whenever OD has plotted on Y-axis, as shown in figure No-4.6.

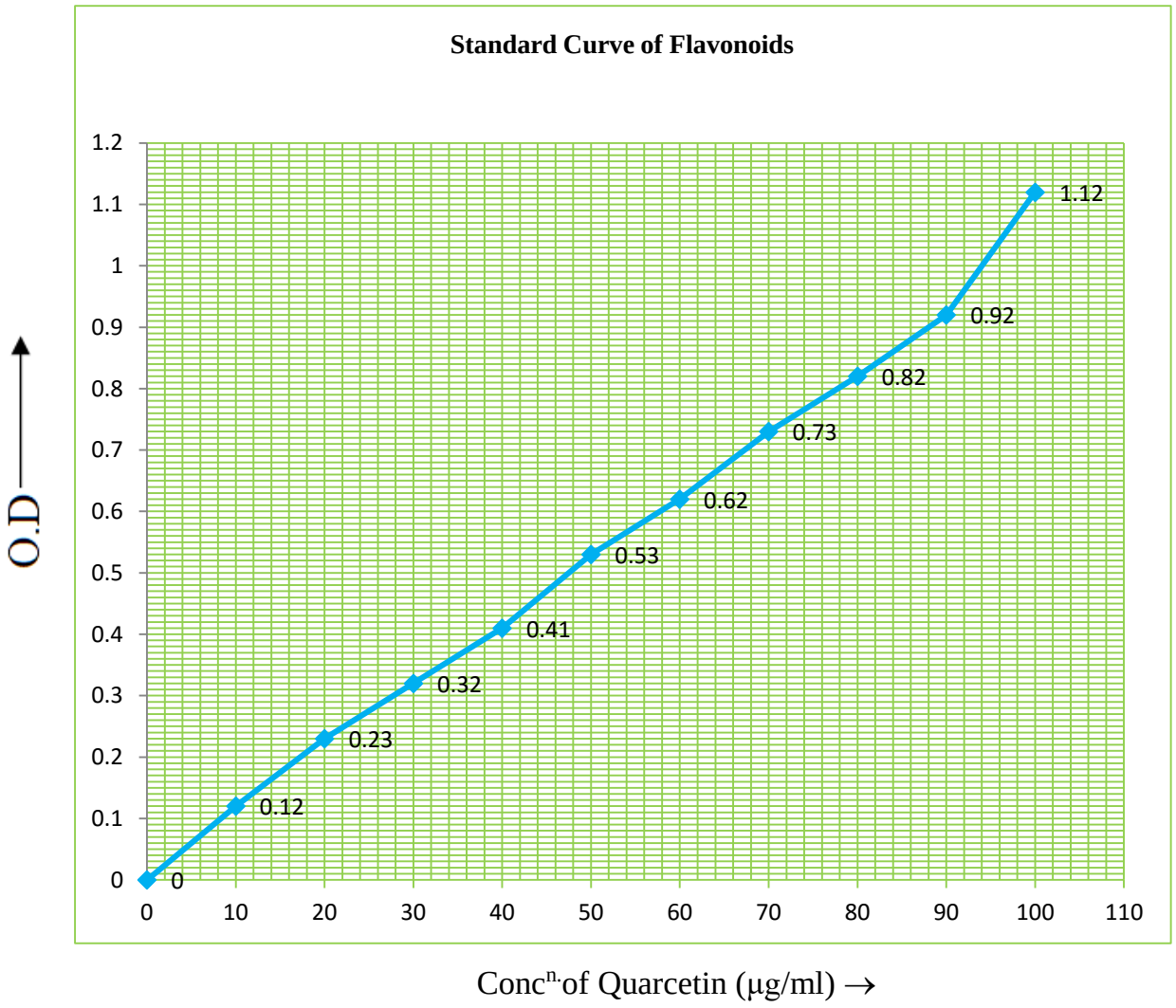


Figure 4.6: Standard curve of flavonoids

Now O.D of each species of Ocimum was taken and it was coincided with standard curve of flavonoids. Concentration of flavonoids in each species has been shown in below table no-4.15.

Table 4.15: Amount of Flavonoid in Ocimum species

Sr.	Plant Species	O.D	Flavonoids in µ g/ml
1	*O.tenuiflorum* L.	0.34	32
2	*O.basilicum* L.	0.28	26
3	*O.gratissimun* L.	0.26	24

This table no. 4.15 reports the optical density (O.D.) values and corresponding flavonoid concentrations (in µg/ml) for three Ocimum species- *O. tenuiflorum* L., *O basilicum* L., *and O.gratissimum* L.. The optical density was measured at an appropriate wave length (commonly around 415 nm for flavonoid estimation), and flavonoid content was calculated based on a standard calibration curve.

- *O.tenuiflorum* L. (O.t) showed the highest O.D. (0.34) and flavonoid content（32 µg/ml), indicating a superior concentration of flavonoids.
- *O. basilicum* L. (O.b) and *O. gratissimum* L. (O.g) showed comparatively lower flavonoid concentrations, 26 µg/ml and 24 µg/ml, respectively.

The trend observed shows a positive correlation between O.D. and flavonoid content, reinforcing the reliability of spectrophotometric quantification in phytochemical studies.

Key Findings:

- **Highest Flavonoid Content in *O. tenuiflorum* L.:**With an O.D. of 0.34 and flavonoid level of 32 µg/ml, *O.tenuiflorum* L. exhibited the highest flavonoid concentration among the tested species, highlighting its potential for antioxidant and therapeutic applications.

- **Lower Levels in *O. basilicum* L. *and O. gratissimum* L.*:*** These species had relatively lower O.D. values (0.28 and 0.26) and flavonoid concentrations (26 µg/ml and 24 µg/ml), indicating lesser but still notable presence of bioactive flavonoids.

- **Spectrophotometric Validation:** The O.D. values correlate proportionally with flavonoid content, confirming the accuracy of this method for quantitative phytochemical screening.

- **Implications for Herbal Use:** Given the differences in flavonoid concentration, species such as *O. tenuiflorum* L. may be preferred for applications requiring strong antioxidant or anti-inflammatory effects.
  From above observation, it was concluded that *Ocimum tenuiflorum* L. (Shyama Tulsi) has highest amount of flavonoids. These phytochemical components like alkaloids flavonoids acts as neuroprotectivs, cardio protective, Anti-inflammatory, Anti-Oxidant, Antiviral and Anti-cancerous, supported by Khanna et al 2003. Glycosides can act as cardio stimulants in cases of cardiac failure (Sood et al 2005)

4.17 Quantitative analysis of Terpenoids: For Quantative analysis of terpenoids, a standard curve has been plotted with different concentration of Linalool against optical density. Following are the observation as shown in Table no. 4.16

Table 4.16: O.D of Linalool in different Concentration

No. of T.T	Vol. of Linalool (ml)	Vol of Chloroform (ml)	Total Vol. (ml)	Vol of Methanol (ml)	OD
B	0	2.0	2	1.5	100
1	0.1	1.9	2	1.5	0.08
2	0.2	1.8	2	1.5	0.16
3	0.3	1.7	2	1.5	0.24
4	0.4	1.6	2	1.5	0.32
5	0.5	1.5	2	1.5	0.40
6	0.6	1.4	2	1.5	0.46
7	0.7	1.3	2	1.5	0.45
8	0.8	1.2	2	1.5	0.62
9	0.9	1.1	2	1.5	0.68
10	1.0	1.0	2	1.5	0.75

Standard Curve of Terpenoids: A standard curve has drawn in which concentration of Linalool has plotted in X-axis and its O.D has been plotted in Y-axis, as shown in below Figure No. 4.7

Terpenoid react with conc.H_2SO4, producing a coloured complex. This indensity of this colour is directly proportional to the terpenoid concentration measured at a specific wavelength at 550 nm. A straight line indicates a good correlation between absorbance and concentration followed by Beer's Law. This standard curve has used to estimate terpernoids in species of Ocimum.

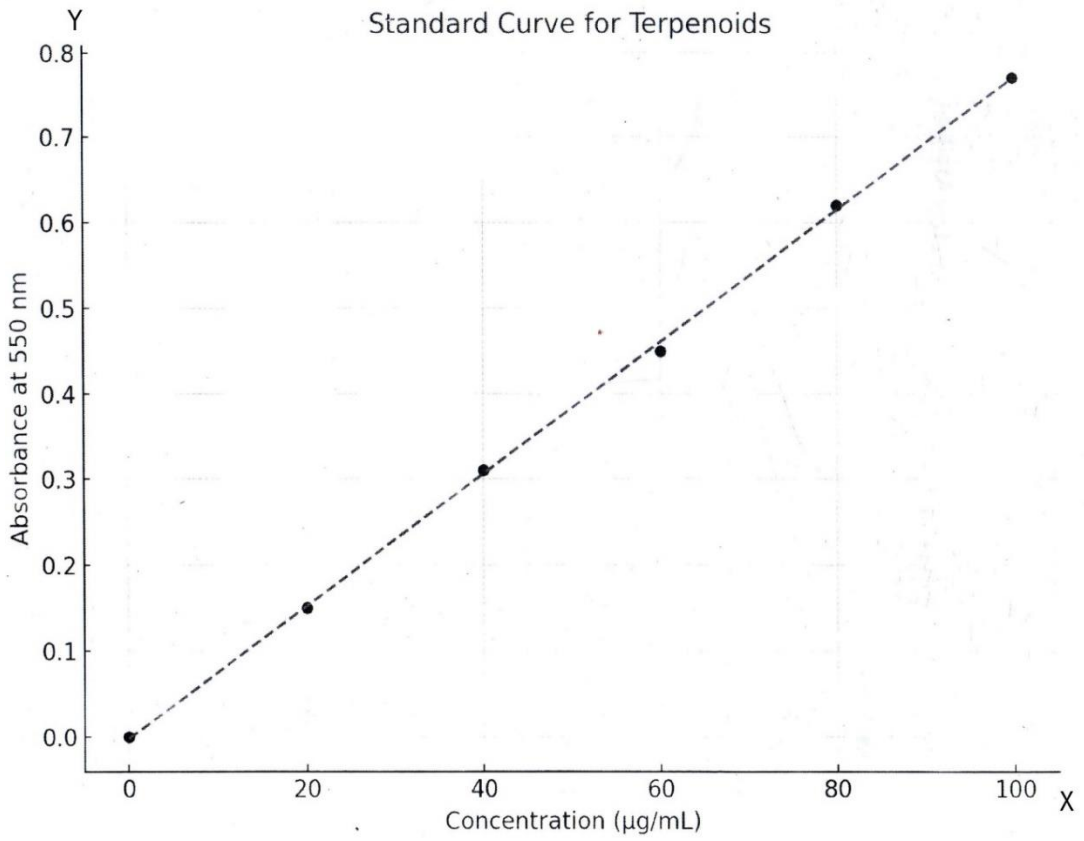


Figure 4.7: standard curve of Linalool

Now O.D of Each species of Ocimum was taken and it was coincided with standard curve followed by concentration of terpernoid in each species has been tabulated as in table no. 4.17

Table 4.17: Concentration of terpenoids

Sl No.	Plant Species	O.D	Terpenoids in µ g/ml
1	*O.tenuiflorum* L.	0.32	40
2	*O.basilicum* L.	0.28	37
3	*O.gratissimum* L.	0.30	38

This table no 4.17, reports the O.D values and corresponding terpenoid concentrations (in µ g/ml) for three Ocimum species- *O.tenuiflorum* L. *O.basilicum* L. *and O.gratissimum* L. The optical density was measured at an appropriate wavelength at 550 nm and terpenoid content was calculated based on a standard curve.

- *O.tenuiflorum* L. showed be hightest O.D (0.32) and terpenoid content (40µ g/ml) indicating in superior concentration of terpenoids.
- *O.gratissimum* L. *followed with (38* µ g/ml)
- *O.basilicum* L. showed comparatively lowest concentration *(37*µ g/ml)

Key Finding:

- **Highest Terpenoid content in *O.tenuiflorum*** L.**:** With an O.D of 0.32 and terpenoid level of (40 µ g/ml), exhibited the highest terpenoid concentration among four species, highlighting its potential for antioxidant and therapeutic application.
- **Moderate terpenoid Yield in *O.gratissimum*** L. ***:*** *O.gratissimum* L. also showed a substantial terpenoid content **(**38µ g/ml), with O.D of 0.30, suggesting a strong phytochemical profile.

Lower levels in *O.basilicum* L.**:** This species has lowest O.D value (0.28) and terpenoid concentration (37µg/ml) indicating lesser but still notable presence of bioactive terpenoid.

4.19 Quantitative analysis of phenol: - For Quantitative analysis of phenol, a standard curve has been plotted with different concentration of Gallic acid against optical density. Following observation as shown in table no. 4.18.

Table 4.18: O.D of Gallic Acid in different Concentration

T.T. No.	Vol of Gallic Acid (ml)	Vol. of D.W (ml)	Conc. Of Gallic Acid (%)	Vol. of F.C Reagent (ml)	Vol. of Na_2CO_3 (ml)	O.D
Blank	00	1.0	00	2	2	94-100
1	0.1	0.9	10	2	2	0.10
2	0.2	0.8	20	2	2	0.22
3	0.3	0.7	30	2	2	0.29
4	0.4	0.6	40	2	2	0.40
5	0.5	0.5	50	2	2	0.48
6	0.6	0.4	60	2	2	0.56
7	0.7	0.3	70	2	2	0.67
8	0.8	0.2	80	2	2	0.76
9	0.9	0.1	90	2	2	0.86
10	1.0	00	100	2	2	0.96

Standard curve of Gallic acid for estimation of phenol: -

A standard curve has drawn in which concentration of Gallic acid has plotted on X-axis and its absorbance (D.O) in Y-axis as shown in below figure no. 4.8

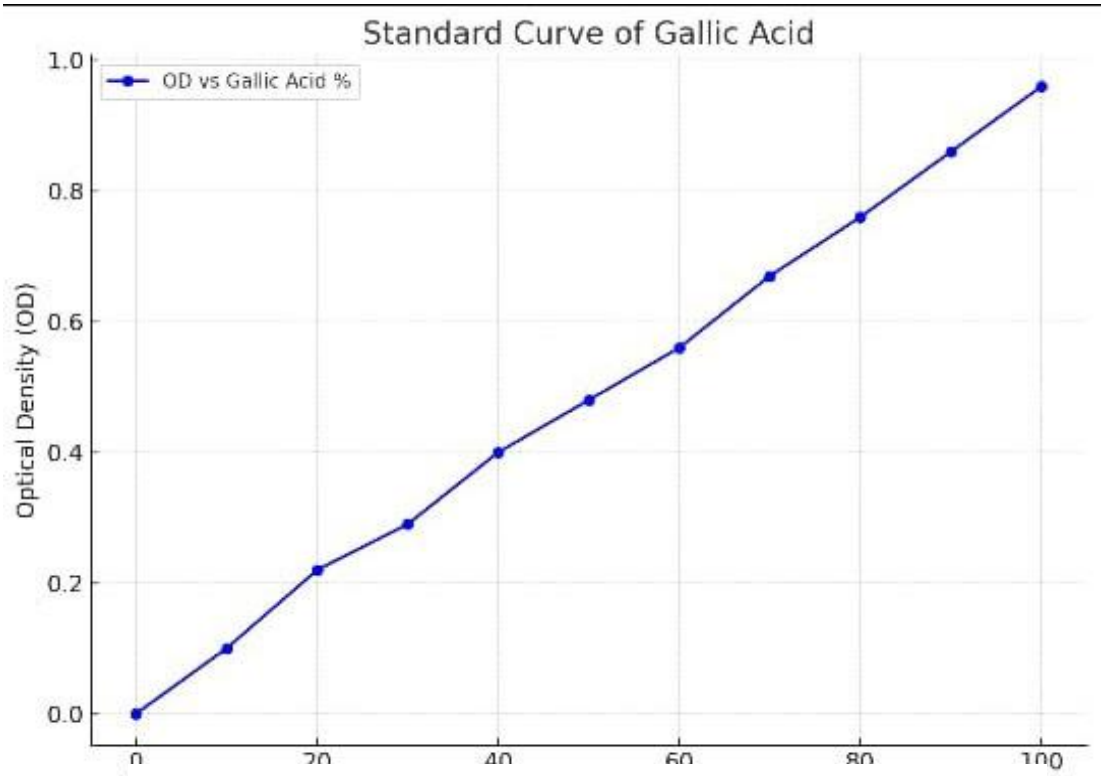


Figure No: -4.8 Standard curve of Gallic acid

A straight line indicates a good correlation between optical density and concentration followed by Beer's Law. This standard curve has used to estimation of total phenolic content (TPC) in species of ocimum.

Now spectrophotometric reading of each species of ocimum has taken and it was coincided with standard curve and its observations were tabulated in below table no. 4.19

Table 4.19: Amount of Phenol in Ocimum species

Sr. No.	Plant Species	O.D	Amount of Phenol µg/ml
1	*O.tenuiflorum* L.	0.280	29
2	*O.basilicum* L.	0.200	22
3	*O.gratissimum* L.	0.230	24

This table no. 4.19, reports the optical density (O.D) values and corresponding phenol concentration in microgram per ml for three Ocimum species- *O.tenuiflorum* L., *O.basilicum* L. and *O.gratissimum* L.. The optical density was measured at an appropriate wavelength at 765 nm, and phenol calculated based on a standard calibration curve.

- *O.tenuiflorum* L. (O.t) showed the highest O.D 0.28 and total phenol content (29μg/ml) indicating a superior concentration of phenolic compound.
- *O.basillicum* L. (O.d) and *O.gratissimum* L. (O.g) showed comparatively lower phenolic concentration, 22μg/ml and 24μg/ml respectively.

Key Finding:

- **Highest phenolic content in *O.tenuiflorum* L.*:*** With an O.D of 0.28 and phenol level of 29μg/ml, *O.tenuiflorum* L. exhibited the highest total phenolic content (TPC) among the tested species.

- **Lower levels in *O.basilicum* L. and *O.gratissimum* L.: -** These species have relatively lower O.D values (0.20 & 0.23) and phenolic concentration are 22 μg/ml and 24 μg/ml respectively, indicating lesser but still notable presence of phenolic compound.

- **Spectrophotometric validation: -** The O.D values correlate proportionally will phenolic content, confirming the accuracy of this method for quantitative phytochemical screening.

- **Implication for Herbal use: -** Phenolic content Ocimum species may be preferred for application requiring antioxidant & anti inflammatory effect.
  From above observation, it was concluded that *O.tenuiflorum* L. has highest amount of phenol among three species, it has anti oxidant and anti inflammatory properties. These compounds can help to protect the body from damaged caused by free radicals, reduce inflammation and potentially lower the risk of chronic diseases like heart disease and cancer.

4.4 Observatoin of standard curve for Cardiac Glycosides estimation:

Table No: 4.20 Absorbance of Digitoxin in different concentration

TT No	Vol. of Digitoxin (ml)	Vol. of Baljet Reagent (ml)	Optical Density
B	**00**	**1.0**	**92-100**
1	**0.1**	**0.9**	**0.24**
2	**0.2**	**0.8**	**0.35**
3	**0.3**	**0.7**	**0.44**
4	**0.4**	**0.6**	**0.53**
5	**0.5**	**0.5**	**0.65**
6	**0.6**	**0.4**	**0.74**
7	**0.7**	**0.3**	**0.83**
8	**0.8**	**0.2**	**0.94**
9	**0.9**	**0.1**	**1.04**
10	**1.0**	**0.0**	**1.17**

4.4.1 Standard curve of Digitoxin:

A standard curve has drawn in which concentration of Digitoxin has plotted on X-axis absorbance (O.D) has plotted on Y-axis as shown in below Figure No. 4.9

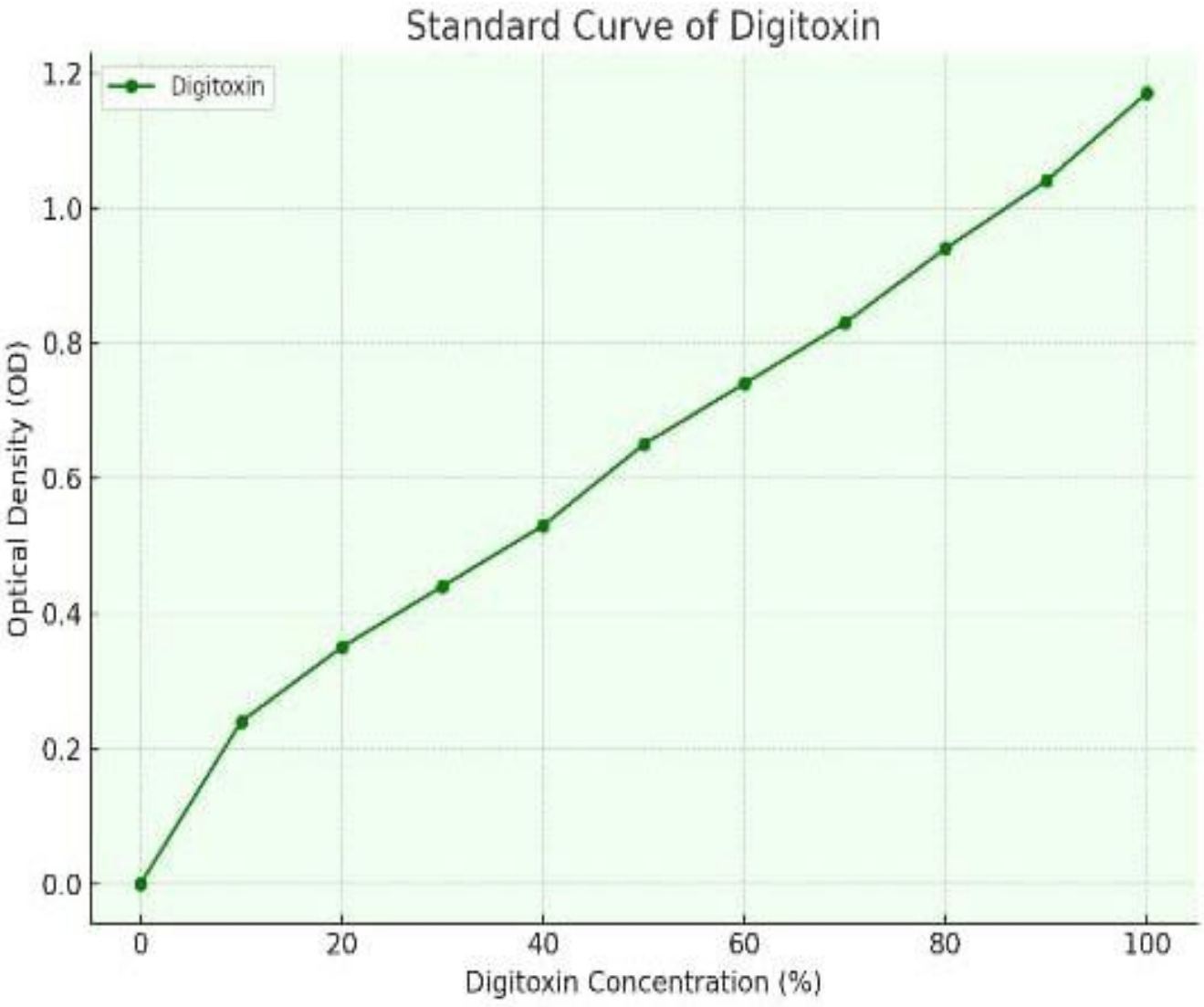


Figure No: 4.9 Standard curve of Digitoxin for estimation of cardiac glycosides

An almost straight line of standard curve indicates a good correlation between optical density (Absorbance) and concentration of Digitoxin followed by Beer's Law. This standard curve has used for estimation of cardiac glycosides in selected species of ocimum.

4.4.2 Quantitative analysis of glycosides: with the help of above standard curve of Digitoxin, concentration of cardiac glycosides has been determined and these values has been tabulated as below table no. 4.21

Table No: 4.21 Concentration of cardiac glycosides

Sr. No.	Ocimum species	O.D	Estimated amount of Glycosides (μg/ml)
1	*O.tenuiflorum* L.	0.36	21
2	*O.basilicum* L.	0.30	15
3	*O.gratissimum* L.	0.36	21

- This table report the optical density (O.D) values and corresponding glycosides concentration in μg/ml for three Ocimum species- *O.tenuiflorum* L., *O.basilicum* L. and *O.gratissimum* L.. The optical density was measured at an appropriate wavelength at 220 nm, and cardiac glycosides calculated based on a standard calibration curve.

- *O.tenuiflorum* L. (O.t) and *O.gratissimum* L. both showed the highest O.D 0.36 and total glycosides (21μg/ml) indicating a superior concentration of glycosides.

- *O.basillicum* L. (O.b) showed comparatively lower glycosidic concentration, 15 μg/ml

4.5 Anaylsis of plant pigments

Analysis of plant pigments in three selected species of Ocimum has also be done by both qualitative and quantitative test through paper chromatography as shown in below figure no. 4.10

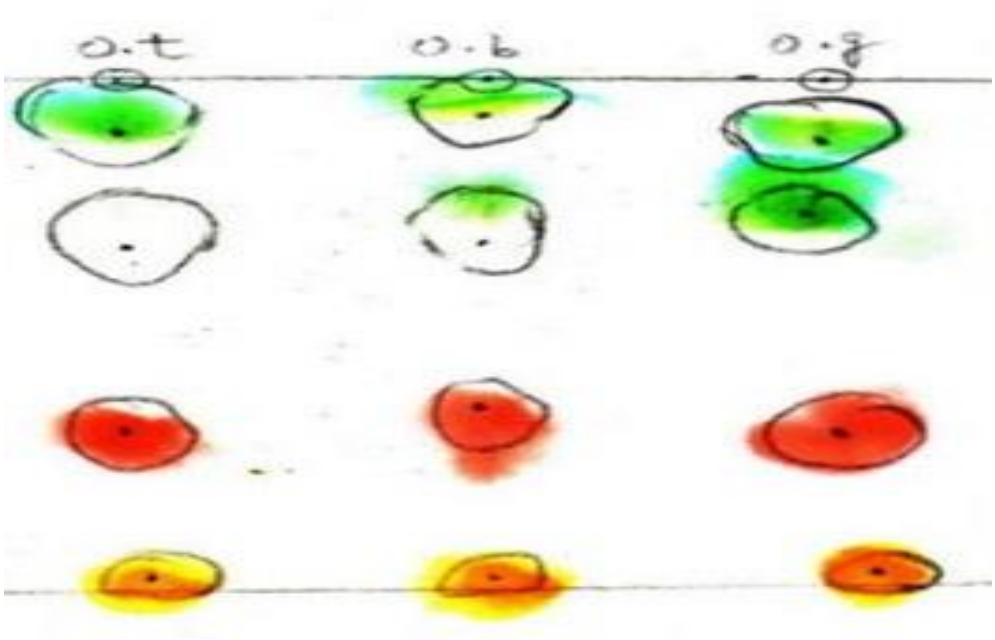


Figure 4.10: Separation of pigment by paper chromatography

Observation:
Color of different patterns were observed which are shown in below table No-22

Table 4.22: R_f Value of pigments

Colour	Pigments	Distance Travelled (cm)			R_f (cm)		
		O.t	O.b	O.g	O.t	O.b	O.g
Yellowish	Carotene	7.6	7.7	7.7	0.76	0.77	0.77
Yellowish Orange	Xanthophyll	5.3	5.5	5.2	0.53	0.55	0.52
Yellowish Green	Ch - a	2.7	2.6	2.0	0.27	0.26	0.20
Green	Ch –b	0.9	0.8	0.8	0.90	0.80	0.80

Leaf extract of all the three species of Ocimum have carotene, xanthophyll, Chlorophyll –a and Chlorophyll – b, which have different mobility. It indicates that chlorophyll 'b' moves slowly and carotene has faster mobility,

Table 4.23 :% T & O.D. of *O.tenuiflorum* L.

Filter	% - Transmittance	O.D. against blank
660nm	10.2	0.98
620nm	11.2	0.91

By Arnon's equation

Chlorophyll a = $(12.7\ OD_{660} – 2.69\ OD_{620})$ x 20mg/Litre

= (12.7 x 0.98 – 2.69 x 0.91) x 20mg/Litre

= (12.446 – 2.4779) x 20mg/Litre

= 9.998 x 20mg/Litre

= 199.962 mg/Litre

Chlorophyll b = $(22.9\ OD_{620} – 4.68\ OD_{660})$ x 20mg/Litre

= (22.9 x 0.91 – 4.68 x 0.98) x 20mg/Litre

= (22.839 – 4.568) x 20mg/Litre

= 16.252 x 20mg/Litre

= 325.052 mg/Litre

Table 4.24: % T & O.D. of *O.basilicum* L.

Filter	% - Transmittance	O.D. against blank
660nm	10.0	1.00
620nm	11.2	0.91

By Arnon's equation

Chlorophyll a = $(12.7\ OD_{660} – 2.69\ OD_{620})$ x 20mg/Litre

= (12.7 x 1 – 2.69 x 0.91) x 20mg/Litre

= (12.7 – 2.477) x 20mg/Litre

= 10.252 x 20mg/Litre

= 205.042 mg/Litre

Chlorophyll b = (22.9 OD_{620} – 4.68 OD_{660}) x 20mg/Litre

= (22.9 x 0.91 – 4.68 x 1) x 20mg/Litre

= (22.839 – 4.68) x 20mg/Litre

= 16.159 x 20mg/Litre

= 323.180 mg/Litre

Table 4.25: % T & O.D. of *O.gratissimum* L.

Filter	% - Transmittance	O.D. against blank
660nm	11.2	0.91
620nm	12.0	0.88

By Arnon's equation

Chlorophyll a = (12.7 OD_{660} – 2.69 OD_{620}) x 20mg/Litre

= (11.557 – 2.367) x 20mg/Litre

= 9.190 x 20mg/Litre

= 183.80 mg/Litre

Chlorophyll b = (22.9 OD_{620} – 4.68 OD_{660}) x 20mg/Litre

= (22.9 x 0.88 – 4.68 x 0.91) x 20mg/Litre

= (22.152 – 4.258) x 20mg/Litre

= 15.894 x 20mg/Litre

= 317.88 mg/Litre

It was observed that *O. basilicum* L. is commonly known as Shyama Tulsi, has the highest amount of chlorophyll by using Arnon's equation through the technique of paper chromatography. Paper chromatography is a technique employed for the separation of chemically closed substances into individual component by their partition coefficient. This is my new finding with

Ocimum species. Comparative chart of Plant Pigments like Chlorophyll a, Chlorophyll b & Total Chlorophyll, as shown in table no-4.26

Table 4.26: Comparative chart of chlorophyll

Ocimum Species	Chlorophyll a (mg/litre)	Chlorophyll b (mg/litre)	Total (mg/litre) Chlorophyll
O. tenuiflorum L.	199.96	325.05	525.01
O. basilicum L.	205.04	323.18	528.22
O. gratissimum L.	183.80	317.88	501.68

- **Chlorophyll b** levels were higher than Chlorophyll a in all cases, consistent with its dominant role in light capture and reaction center activity in photosystems.

Table 4.26 reports that Chlorophyll b/a ratio remained relatively stable across species and locations, but *O. gratissimum* L. *and O. basilicum* L. exhibited slightly higher absolute values for both pigments, indicating enhanced chloroplast development and photosynthetic potential. *O. basilicum* L. has the highest amount of Chlorophyll.Total amount of chlorophyll 528.22 mg/litre in *O. basilicum* L., this indicates that *O. basilicum* L. has capacity to catch the light energy of the sun and convert water and CO_2 to produce glucose and oxygen. It is a green pigment located in the chloroplast that are site for process of photosynthesis. High conduct of chlorophyll provide increased fibre and antioxidant, which may benefit in cancer prevention, supported by Jared Meacham 2024.

4.6 Oil extraction from the leaf of Ocimum species:

Essential oil are extracted through Clevenger apparatus and oil components by using two types of solvent Benzene & Methanol in the

ratio of 10:1 and Toluene + Ethyl acctate + Acetic acid in the ratio of 8:2:05.

Observation: After ½ hour plate was removed from incubator because spot was developed and R_f value was determined by using the formula

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Table 4.27 :R_f value of Ocimum species through TLC

Plant Species	R_f Value			
	Solvent Benzene + Methanol (10:1)		Solvent used TEA 8:2:0.5	
	Spot 1 (cm)	Spot 2 (cm)	Spot 3 (cm)	Spot 4 (cm)
O. tenuiflorum L.	0.62	0.42	0.58	0.67
O. basilicum L.	0.64	0.43	0.58	0.66
O. gratissimum L.	0.63	0.45	0.56	0.68

According to R_f value Linalool, Geraniol, Eugenol and Thymol have been detected. After determination of R_f value, it was found that there may be Linalool and Geraniol because the standard R_f vaue of Linalool is 0.60 (± 0.2) and Geraniols 0.40 to 0.45 for Spot 1 and Spot 2 respectively in plate 1. But in plate 2, R_f value was determined & it was found the R_f value in Thymol and Eugenol because standard R_f value of Thymol and Eugenol are 0.58 and 0.66 respectively, it may be Thymol and Eugenol, as shown in table No-4.29. Overall it was identified that spots 1, 2, 3 and 4 are Linalool, Geraniols, Thymol & Eugenol respectively.

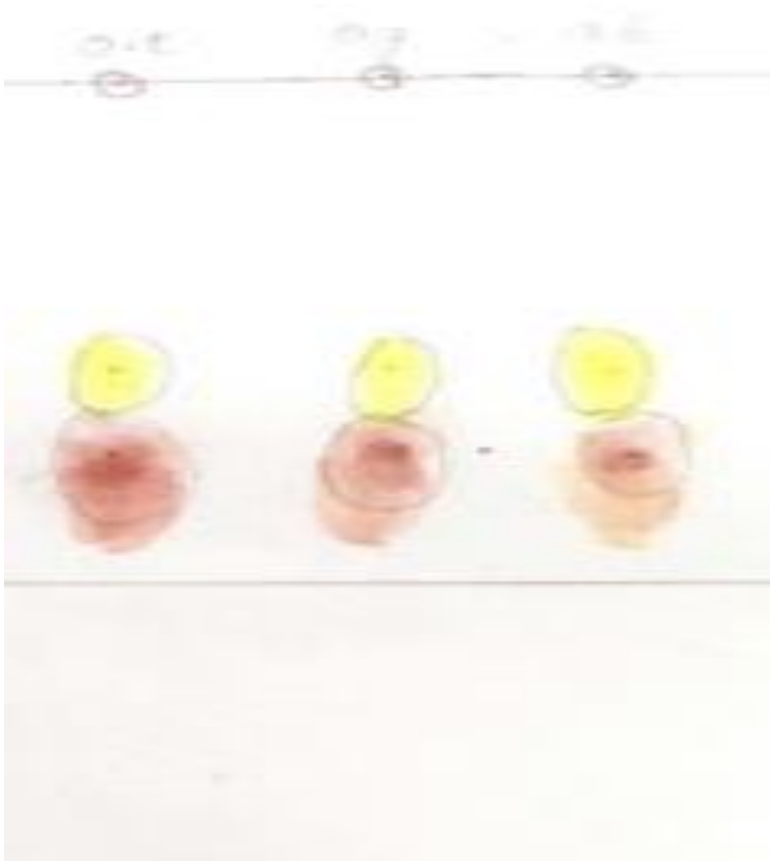


Figure 4.11 : TLC of extracted oil showing Linalool (L) & Geraniol (G)

Figure 4.12: TLC of extracted oil showing Thymol (T) & Eugenol (E)

LINALOOL: TLC Spot Colour - Brown

Properties: It acts as antimicrobial, anti-cancerous, antioxidant and antifungal agent.

Used: (a) It is used in medicine.

(b) It is used in the preparation of hygiene product like cleaning agents (Soaps &detergent, Shampoo) as a scent.

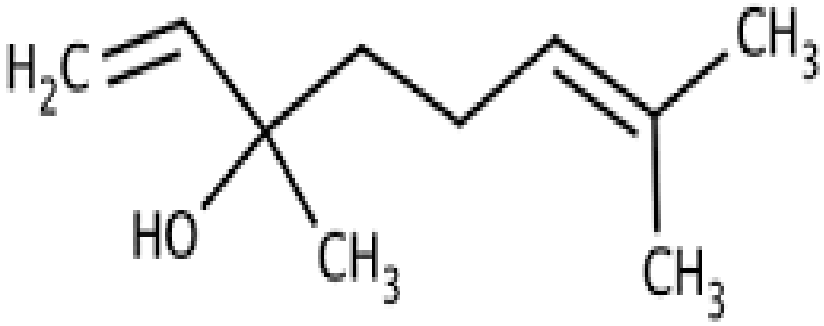


Figure 4.13: Structure of Linalool ($C_{10}H_{18}O$)

GERANIOL: TLC Spot Colour - Yellow

Properties: It is an antimicrobial, anti-inflammatory, antioxidant, anticancer and Neuro-protective.

Used: (a) It is used in medicine

(b) It is used in food fragrance and cosmetic industry.

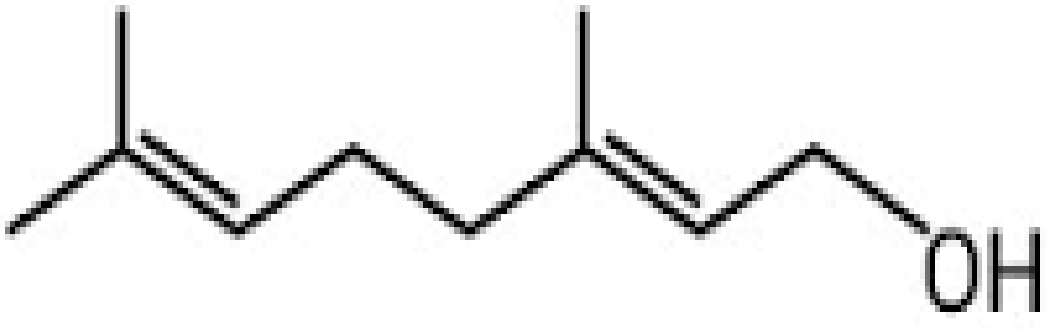


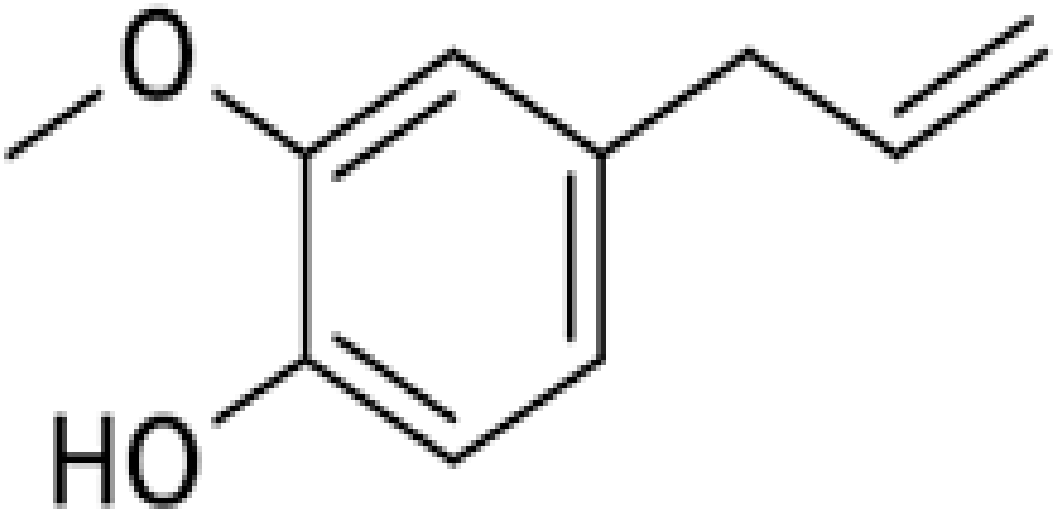
Figure 4.14: Structure of Geraniol ($C_{10}H_{18}O$)

EUGENOL: TLC Spot Colour – Dark Brown

Properties: Eugenol is weakly acidic phenolic compound.

Used: (a) It is used in antitumor, antimicrobial medicine.

(b)It is also used for anti-inflammatory and antioxidant medicine.



T

Properties: It is phenolic compound. It inhibits the growth of microorganism in urine specimen.

Used: (a) It is used in medicine for antioxidant, anti-infective and anti-inflammatory.

(b) It is used in tooth paste, mouth washes, chewing gum.

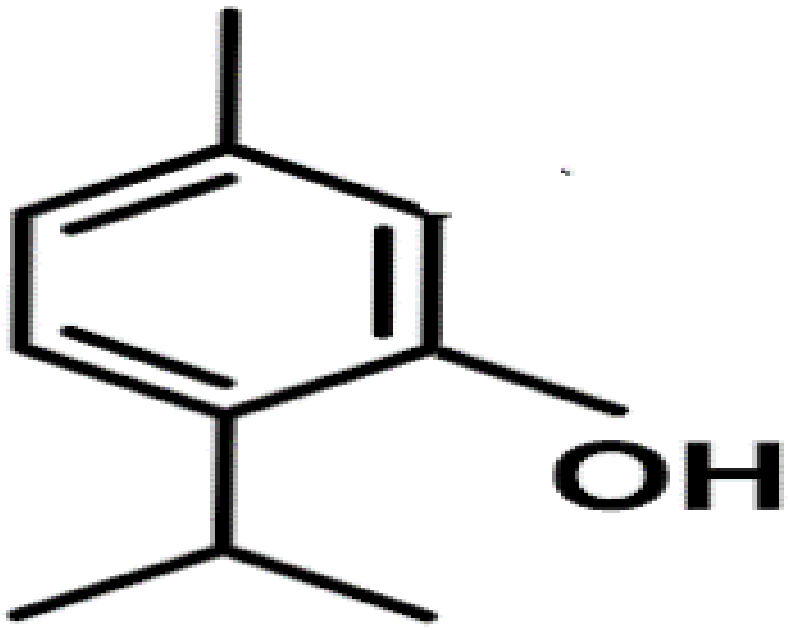


Figure 4.16: Structure of Thymol ($C_{10}H_{14}O$)

Linalool acts as antimicrobial, anticancerous, antioxidant and antifungal agent so it is used in medicine, food fragrance and cosmetic industry. Eugenol is phenolic compound, it inhibits the growth of microorganism in urine specimen, over all it may concluded that phytochemicals from the plant of *O. tenuiflorum* L. can be used in various diseases. The herbs are cheap, available in large quantity around us and they pose no danger to be living organisms, the environment and the consumers and hence greatly helpful for living organism.

B. Molecular Characterization of Ocimum Species.

Molecular characterization determines the genetic characteristics of the organism in plants or animals. The basic principle or extraction that all other components of the chromatin are removed leaving behind the DNA. After isolation of DNA, its quality has been checked by spectrophotometric method. After isolation of DNA, it was ready for agrose gel electrophoresis. Agarose is a high molecular weight polysaccharides, extracted from the wall of certain marie algae, is an alternating co-polymer of 1,3-linked β-D galactose and 1,4-Linked 3,6-anhydro α-L-galactose. Agarose gels have become the work house of the molecular biology work because they are easy to cast and quick run. By using different concentration of agarose, it is possible to resolve a wide range of DNA molecuses. In gel, the electrophoretic mobility of a macro molecule is determined by the volume fraction of pores within the gel that the macromolecules can enter. Since small DNA molecule can enter in more number of pores, it will migrated through the matrix. Following of the figure (Figer No-4.17) of gel electrophoresis there are 3 lanes, numbered as 1, 2 & 3 for O.t, O.b & O.g respectively. There were no smearing found below the orange band of DNA, so it is clear that the quality of DNA is good.

In this work it has been found that optical density of *O.tenuiflorum* L., *O.gratissimum* L. & *O.basilicum* L. are 3.32, 3.32 & 3.30 respectively from which DNA concentration in nanogram per micro liter has been found. 166,

166 & 165 respectively. This method is also supported by sadaf Quraishi et al 2011 who found 200 nanogram per micro liter in *O.gratissimum* L., 280 nanogram per micro liter in *O.americanum* L., so it can be concluded that Shyama Tulsi (*O.tenuiflorum* L.) has higher content of DNA than *O.gratissimum* L.

3.10.3 DNA – Quantification

There are two widely used methods to quantitate DNA (Sambrook et al, 1989). One is the spectrophotometric measurement of the amount of UV-radiation absorbed on the basis of DNA and other in comparison of fluorescent intensities of unknown and standard DNA sample by running agarose gel and then observing under UV-light. In this work spectrophotometric method has been used.

Spectrophotometric method: - In this method following formula is used

$$\text{DNA concentration } (\mu g/\mu l) = \frac{O.D260 \times dilution\ factor \times 50}{1000}$$

Table 4.28: DNA Quantification

S/No.	**Ocimum Species**	**O.D**	**DNA (ng/µl)**
1.	*O. tenuiflorum* L.	3.32	166
2.	*O. gratissimum* L.	3.32	166
3.	*O. basilicum* L.	3.30	165

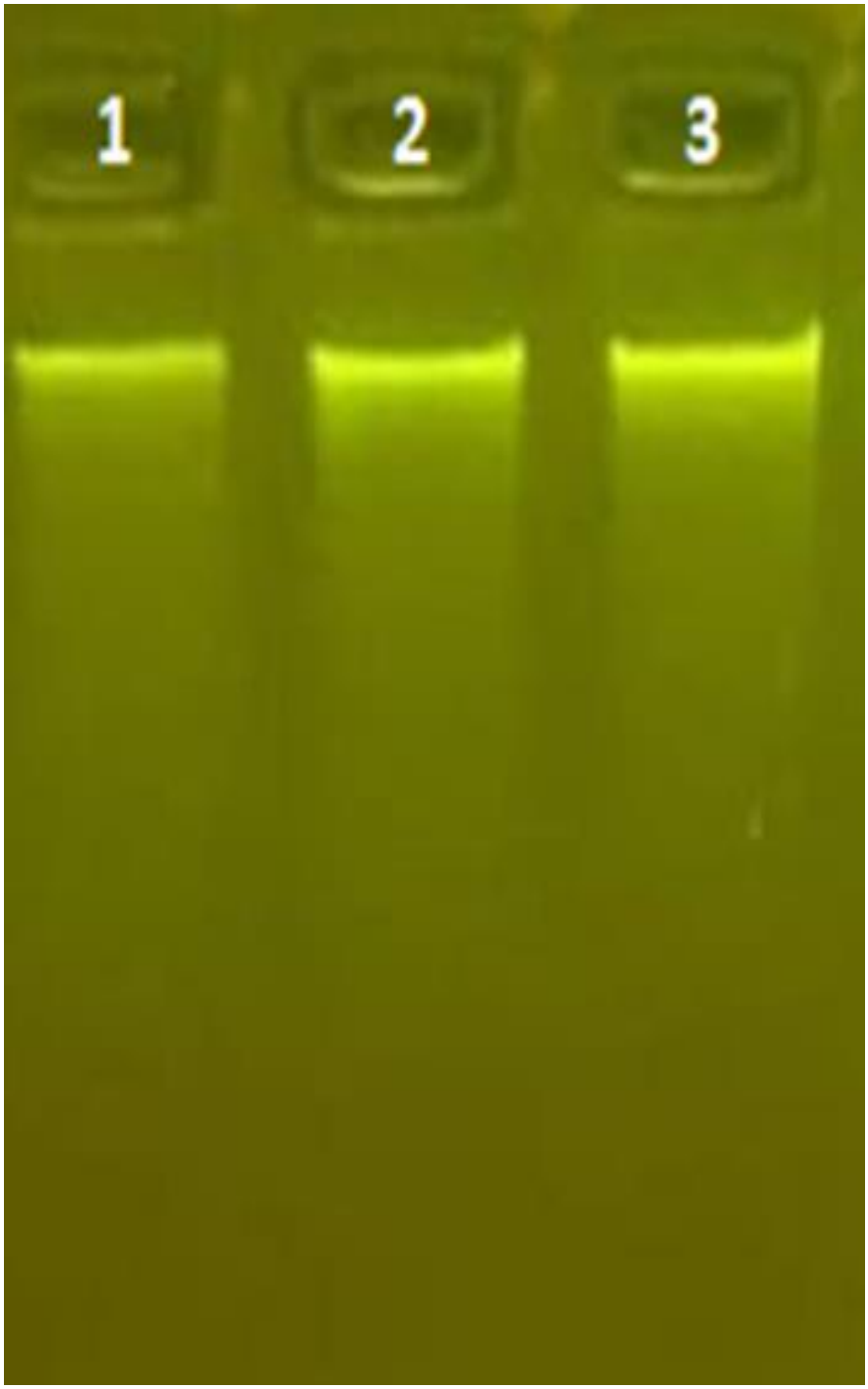


Figure 4.17: Gel Electrophoresis showing DNA bands for
1. *O. tenuiflorum* L. 2. *O. gratissimum* L. 3. *O. basilicum* L.

After quantification of DNA & agarose gel electrophoreses, PCR programme was done to enhance the quantity or to amplify the DNA. PCR reaction used two oligonucleotide primers that hybridize to opposite strand and flanked the target DNA sequence to be amplified. A heat stale DNA polymerase such as Taq Polymerase catalyses the elongation of the primers. Template

denaturation, primers and extension of the annealed primers by the polymerase results in exponential accumulation of a specific DNA fragment. The primer extension products synthesized in a given cycle can serve as the template in the next cycle.

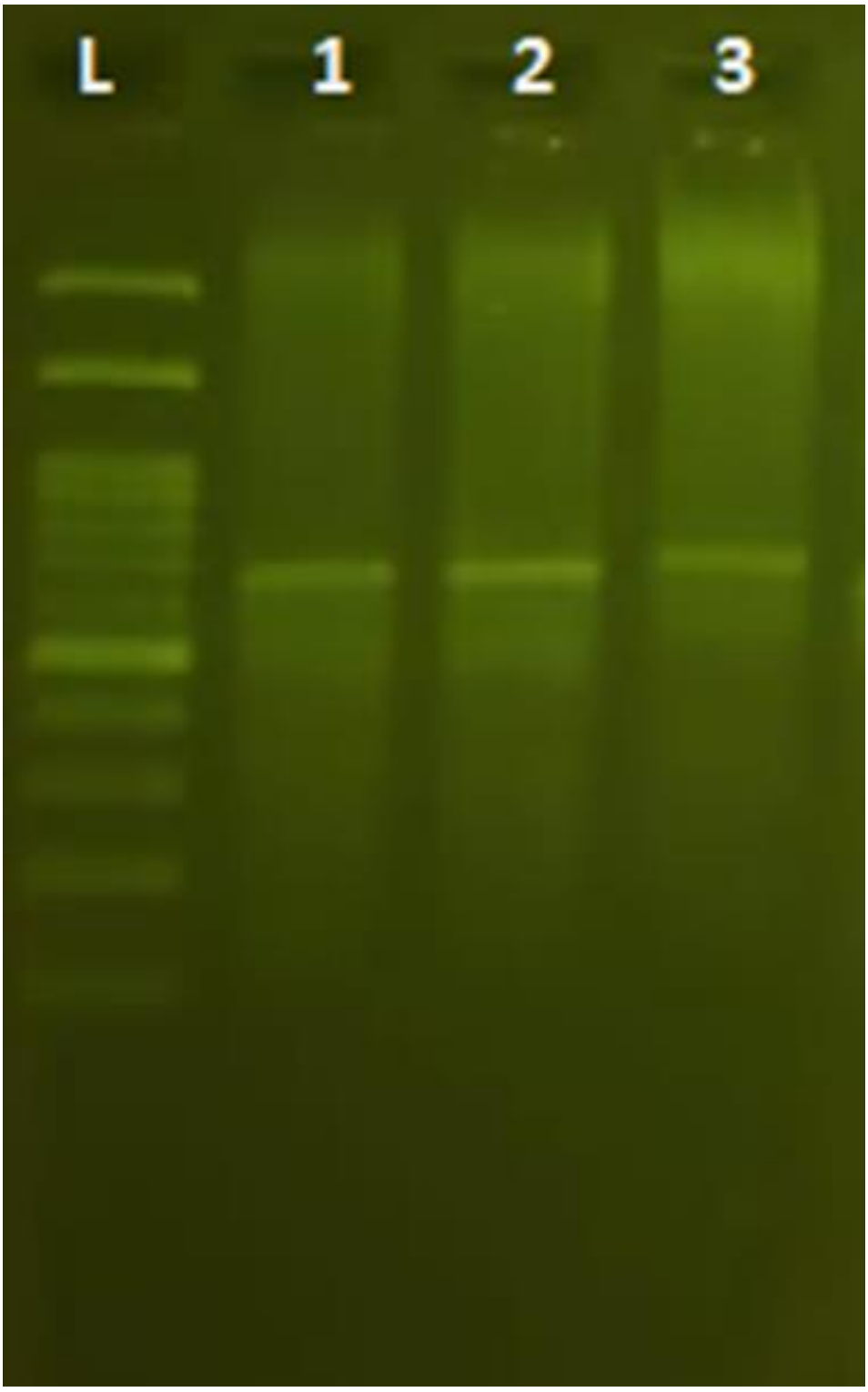


Figure 4.18: DNA Bands after PCR

The number of target DNA copies approximately double in every cycle. After completion of the PCR programme, the PCR products were checked on 2% agarose for the amplification as in below figure-4.18, there are 4 lanes in the gel symbolized as L, 1, 2 & 3 stand for Ladder. *O.tenuiflorum* L., *O.gratissimum* L. & *O.basilicium* L. respectively

After knowing the concentration of DNA, it was ready for Sanger Sequencing where gene sequencing are observed as Electropherogram supported by Ahmad Bilal. 2020

Sanger sequencing has been done to know the sequencing of nitrogenous base, where gene sequencing was observed as electropherogram. In this electropherogram all the four nitrogeneous bases indicated in different colour. Red peak stand for thymine (T), green peak stand for adenine (A), blue peak for cytosine (C) and brown peak stand for guanine (G). The height of the peak reflects the signal strength.The signal strength shows on x-axis from 100 to 1000 RFU.

The position of the peak on the x-axis indicates the location of the nucleotide in the DNA sequence. The sequence characterizes by having multiple peaks. It was observed that out of 320 nucleotides, there are A=92, T=91, G=70 and C-67 (Fig. No.4.19) in *O. tenuiflorum* L. In *O.basilicum* L. A=90, T=90, G=72 and C=68 (Fig No. 4.20) whenever, in *O.gratissimum* L. A=81, T=88, G=75 and C=76 (Fig No. 4.21) peaks were found. It is also tabulated as below.

Highest length of peak up to 320 nitrogeneous bases and which touch 800 RFU are as G=6, T=1, C=0 and A=0 in species of *O.basilicum* L. G=22, T=4, C=8 and A=13 peaks in species of *Ocimum gratissimum* L.. And lastly in *Ocimum tenuiflorum* L. there are 79 bases touch the range of 800 RFU in which G=16, T=25, C=19 and A=17, after this observation of Sanger sequencing it was Concluded that *O. tenuiflorum* L. (Shyama Tulsi) has

highest number of Nitrogeneous bases which has longest peak, crosses 800 RFU,(This is my new finding among Ocimum species)

Table 4.29: Number of Nitrogeneous bases after Sanger sequencing upto 320 bases

Ocimum Species	Nirogeneous bases				Total No. Peaks
	A	T	G	C	
Ocimum tenuiflorum L.	92	91	70	67	320
Ocimum basilicum L.	90	90	72	68	320
Ocimum gratissimum L.	81	88	75	76	320

Observed Result and Data Interpreation for Sanger sequencing of Ocimum species

The DNA sequence chromatogram shown in Figure No-4.19 represents the Sanger sequencing output of the rbcl gene region amplified from *Ocimum tenuiflorum* L.The sequencing was performed using capillary electrophoresis on an Applied Biosystems 3730x1 Genetic Analyzer, and the data were analyzed using Sequence Scanner Software v2.0. The electropherogram illustrates the typical fluorescent signal peaks for each nucleotide base-adenine (green), thymine (red), cytosine (blue), and guanine（brown). The clarity and sharpness of the peaks, particularly in the central regions of the chromatogram (e.g., base positions 50 to 300), indicate high-quality sequence reads with minimal background noise or overlapping signals. This confirms that the PCR amplification and sequencing reaction were successful and reliable for downstream bioinformatics analysis.

Peaks represent the four DNA bases: adenine (green), thyminc (red), cytosin （blue), and guanine (brown). The sequencing quality is notably high across the majority of the fragment, with peak heights averaging consistently around 600-800 fluorescence units, and clear base separation from approximately position 10 to beyond 370.

The average signal strengths for each base (G:197, A:191,T:188,C:167) reflect balanced base incorporation and efficient dye termination chemistry.The basecall confidence (QVs) is high throughout the electropherogram, as shown by the prevalence of "pure base QVs" in the blue bar at the bottom. This further validates the lack of background noise or ambiguous peaks, confirming the mono-template origin of the DNA.

The sequence is suitable for:

- **Taxonomic identification and barcoding** of *O. basilicum* L.
- **Molecular phylogenetic analysis** among species of *Ocimum,*
- **Database submission** for future referencing or comparison.

In conclusion, Figure No- 4.20 clearly demonstrates that the Sanger sequencing of the rbcL gene region in Ocimum basilicum was successful, yielding a clean, interpretable, and publication-quality sequence. This validates the sample's authenticity and supports its use in downstream genetic analysis and comparative studies with other Ocimum species such as *O. tenuiflorum* L. (refer to Figure No- 4.19).

Figure No-4.21 depicts the Sanger sequencing chromatogram of the rbcL gene fragment of *Ocimum gratissimum* L. performed using an ABI 3730x1

DNA analyzer and analyzed via Sequence Scanner Software v2.0. This electropherogram reflects the nucleotide sequencing output through the detection of fluorescently labeled dideoxy nucleotides, enabling precise

determination of the base order.The color-coded peaks correspond to the four DNAbases-adenine (grecn), thymine (red),cytosine (blue), and guanine (brown) and read from left to right across multiple lines for continuity of sequence.

The electropherogram shows a moderate-quality read with clearly distinguishable base peaks up to around the 3900th base position. However, compared to *O.basilicum* (Figure No- 4.20), the signal strength (G:70, A:60, T:60, C:56;)is relatively lower, indicating a weaker overall fluorescence intensity.Despite this, most of the base calls remain accurate and unambiguous, with a considerable proportion of "pure base QVs" as highlighted in the quality summary bar at the bottom of the figure.

Notably:

- From position 100 to approximately 2900, the peaks are distinct and well-resolved, indicating reliable base calling.
- Beyond position 3000, some peak overlaps and reduced sharpness are observed, likely due to signal decay or dye terminator depletion, a common artifact in longer reads.
- Despite minor signal noise and peak broadening in the later part of the sequence, the majority of the electropherogram remains interpretable and usable for downstream applications.

The sequence is deemed adequate for:

- DNA barcoding and phylogenetic placement of *O. gratissimum* L.
- Comparative sequence analysis with other *Ocimum* species (such as *O. basilicum* L. and *O. tenuiflorum* L.),
- Molecular authentication studies within the genus *Ocimum*.

In conclusion, Figure No- 4.21 confirms the successful amplification and sequencing of the *rbcl* gene in *Ocimum gratissimum* L. with acceptable

sequence integrity for bioinformatics analyses despite some minor signal degradation in the later regions. The data contributes significantly to the molecular characterization and species-level identification of *O. gratissimum* L. within the broader framework of Ocimum biodiversity studies.

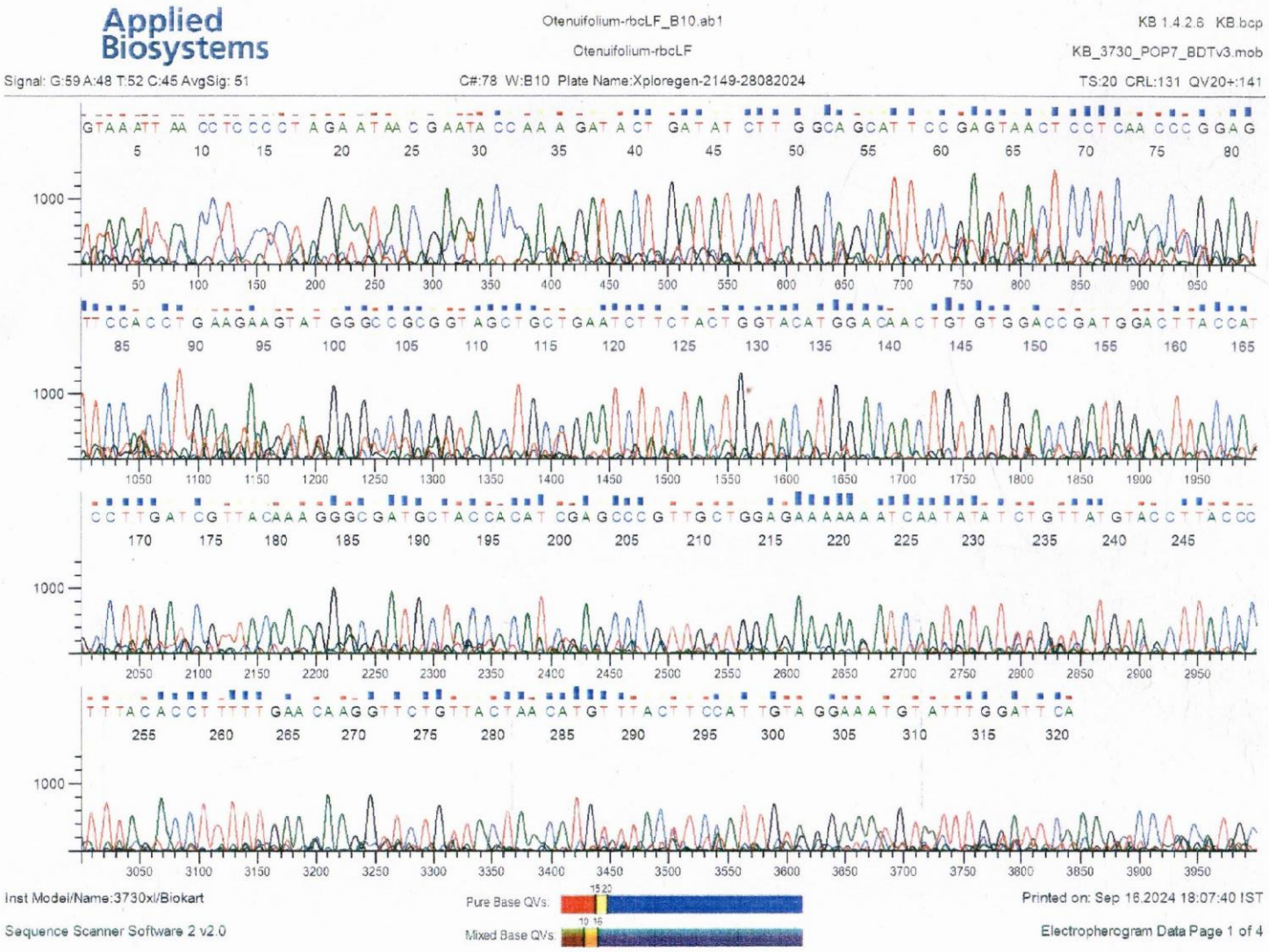


Figure 4.19: Electropherogram for *O.tenuiflorum* L.

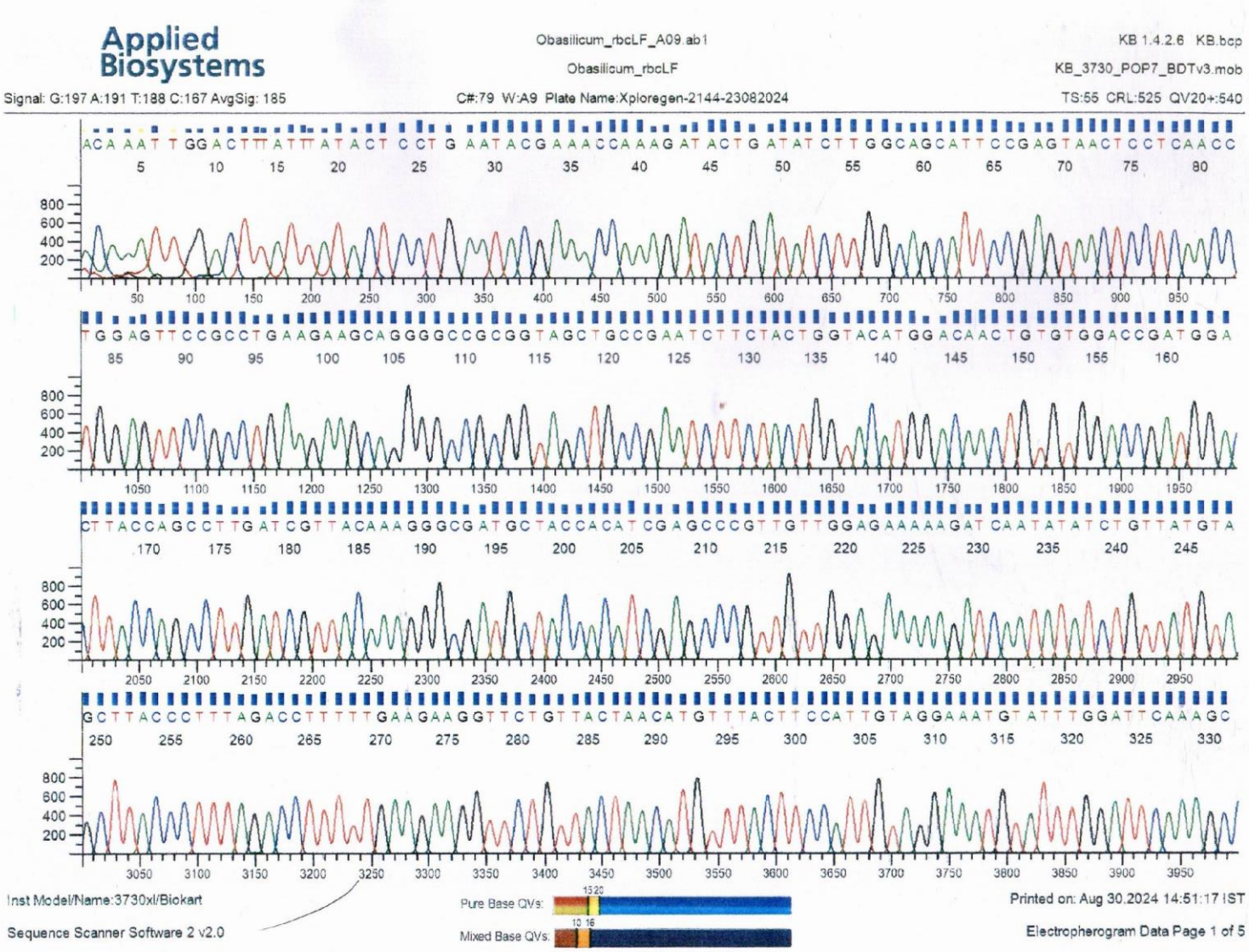


Figure 4.20: Electropherogram for *O.basilicum* L.

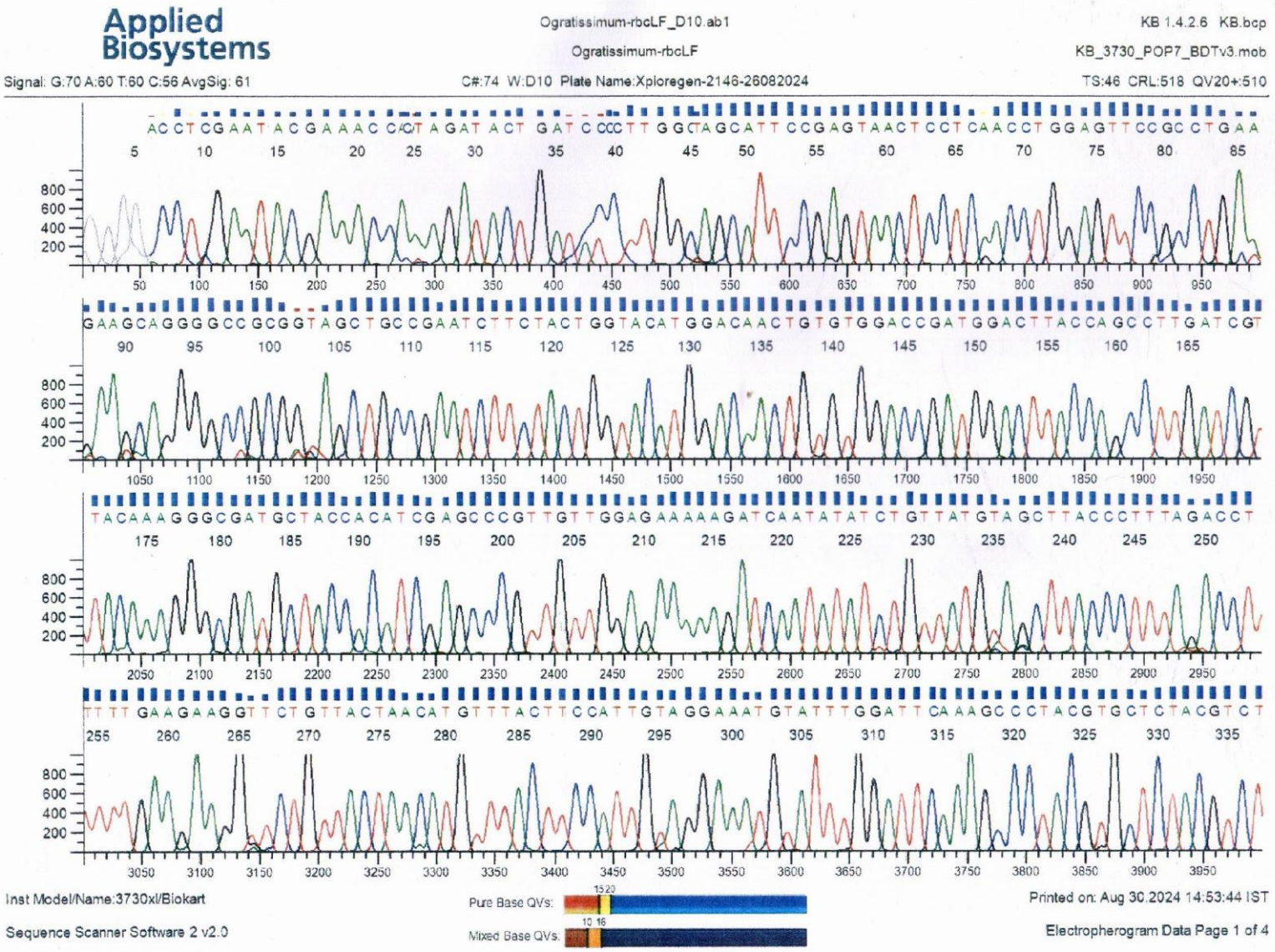


Figure 4.21: Electropherogram for *O.gratissimum* L.

CHAPTER-5

CONCLUSION

CONCLUSION

Ocimum is a significant candidate in studying and discovering novel bioactive compounds and their usage in treating incurable diseases, as access to naturopathy has risen globally and a huge number of researchers are in the works to present innovative natural goods. Exploration of plant chemical components and pharmacological screening will thereby lay the groundwork for the development of novel life-saving medications. *O.gratissimum* L. *O.tenuiflorum* L. and *O.basilicum* L. were the most investigated species with well known traditional applications and ethnopharmacological importance according to our findings. Several classes of compounds have been found, containing flavonoids and phenolics, lignins, coumarins, terpenoids, and fatty acid derivatives, with flavonoids and terpenes being the most common categories of compounds responsible for the majority of the bioactivities. Essential oils derived from a variety of Ocimum spp. provided good supplies of natural eugenol and were widely used in the pharmaceutical, cosmeticsand food sectors Preclinical and clinical research have shown that *O.tenuiflorum* L. has immunomodulatory and adaptogenic properties, which may provide more confidence on the effectiveness and safetyof Ocimum as a herbal supplement. So that Ocimum plants can contribute significantly to the economy and health care.

It was concluded that *Ocimum tenuiflorum* L.（Shyama Tusli) is very useful among three selected species of Ocimum. For diabatic and Arthritis patient *Ocimum tenuiflorum* L. (Shyama Tulsi) is more benefited for treatment of cough, to reduce swelling, to cure diabetes mellitus, dysentery, loose motion and can be used in unani or herbal or Ayurvedic medicine. *Ocimum tenuiflorum* L. has highest content of protein so it is also very useful for medicine, as both *Ocimum tenuiflorum* L. can be used as antimicrobial diseases caused by various palhogens.It was also concluded that *O.tenuiflorum* L. has a highest amount of phenol among four selected species of Ocimum so it may have antioxidant and antiflammatory properties. These compounds can helps protect the body from damaged caused by free radicals,

reduce inflammation, and potentially lower the risk of chronic diseases like heart diseases and also cancer.

It was also concluded that *O.tenuiflorum* L. and *O.gratissimum* L. have highest amount of cardiac glycosides among among three selected species of ocimum. Amount of cardiac glycosides was identified using standard curve with standard glycosides solution like Digitoxin. Digitoxin is cardiac glycosides derived from leaf of *Digitalis Purpurea,* commonly known as Foxglove leaves that treat heart failure and chronic cardiac insufficiency. It may work by blocking the sodium potassium ATpase in the plasma membrane which increase intracellular calcium and sodium levels.This will lead to increased mycocardial contraction and a slower heart rate.Extract of *Ocimum tenuiflorum* L. may be useful against fever, ulcer, cough, arthritis, antimicrobial infection, antifungal etc. On the basis of molecular characterization, it was concluded that *O.tenuiflorum* L. and *O.gratissimum* L. have the same amount of DNA that is 166 ng/µl whenever in *O.basilicum* L. have same amount of DNA that is 165 ng/µl. the amount of DNA in Tulsi leaves indicate the over all genetic materials present.

After Sanger sequencing, it was concluded that *O.tenuiflorum* L. (Shyama Tulsi) has highest No of Adenine upto 320 ng/µl among for selected species of Ocimum it indicate the molecules responsbule for storing and transmitting genetic information. Over all it was also concluded that peak of Guanine has longest peak among for bases 800 RFU after this study it is assumed that the extract could be used for new formulations of medicine.

Table 4.30 : Result of all experiments, at a glance.

Charecterization	Experiment	Method used	Amount (μg/ml) (+ indicates available)			Apparatus/ Equipment used
					12	
PHYTOCHEMICAL & MOLECULAR	**Primary Metabolites**					• Centrifuge Machine • Colorimeter • Chromatography paper/Jar • Clevenger apparatus • Electrophoresis apparatus • Glass wares • Incubator • Monopan balance • Microwave oven • Oven • Spectrophotometer • Soxhlet apparatus • Sanger Sequencing machine • TLC apparatus • Thermal cycler (PCR Machine) • UV illuminator
	Protein	Lowry et al method	40	36	38	
	Sugar	Nelson method	2.58	2.88	2.76	
	Secondary Metabolites					
	Alkaloids	Gravimetric method	118	96	110	
	Flavonoids	$AlCl_3$ method	32	26	24	
	Glycosides	Through Digitoxin	21	15	21	
	Terpenoids	Through Linalool	40	37	38	
	Phenol	Through Galic Acid	29	22	24	
	Plant Pigments					
	Chlorophyll a	By Arnon's equation	199.96	205.04	183.88	
	Chlorophyll b	By Arnon's equation	325.05	323.18	317.88	
	Total Chlorophyll	By Arnon's equation	525.01	528.22	501.76	
	Oil Extraction					
	Linalool	TLC method	+	+	+	
	Geraniol	TLC method	+	+	+	
	Thymol	TLC method	+	+	+	
	Eugenol	TLC method	+	+	+	
	Molecular Exp.					
	DNA	CTAB method	166 ng/μl	166 ng/μl	165 ng/μl	
	Adenine	Sanger Sequencing	92/320 Peaks	90/320 Peaks	81/320 Peaks	
	Thymine	Sanger Sequencing	91/320 Peaks	90/320 Peaks	88/320 Peaks	
	Guanine	Sanger Sequencing	70/320 Peaks	72/320 Peaks	75/320 Peaks	
	Cytosine	Sanger Sequencing	67/320 Peaks	68/320 Peaks	76/320 Peaks	

CHAPTER-6

Future Prospects

FUTURE PROSPECTS

- Tusli or Ocimum species, holds significant future promise due to presence of primary & secondary metabolites and it offers a wide range of possibilities for future applications in various sectors including pharmaceuticals, foods and cosmetics.

- Tusli farming can be a lucrative agribusiness opportunity, especially raising demand for natural organic product.

- Processing Tusli into essential oils, extracts and other products can create additional revenue streams for farmers and business.

- Tulsi's spiritual and cultural significance in many cultures, particularly in India, adds another dimesion to its future prospects. Connecting people to nature and tradition.

- Government's iniatives and funding for sustainable cultivation and market expension can further boost the global demand for Tulsi.

Tulsi, a versatile plant, plays a crucial role in various economic sectors contributing to the prosperity of communities that cultivated it. It has gained importance in the herbal and pharmaceutical industries due to its diverse medicinal properties. After Sanger sequencing it was found that the longest peak is of mostly "Guanine." Future studies will likely focus on harnessing the unique propersites of Guanine for various biotechnological and medicinal applications. For example, researcher are exploring the use of Guanine, which for anticancer therapists and as tools for regulating gene expression.

CHAPTER-7

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Annexure I

List of Publications

Sr.	Topic	Journal Name	National/International
1	A Glance at the phytochemical Ethno-pharmacological Understanding of four Ocimum species	Current Pharmaceutical Biotechnology, 2023, Vol 24	International 2023 Vol 24 (ISSN 1389-2010) UGC care list
2	Phytochemical screening and Environmental Benefit of Tulsi plant	International Journal of Innovative science and research technology	International (ISSN 2456-2165) UGC care list
3	Comparative study of alkaloids & Flavonoids in selected species of Ocimum in and around of Munger District	International Journal of creative research Though	International Feb 2024 Vol 12 (ISSN 2320-2882) UGC care list
4	Comparative study of DNA Quantification of four selected species of Ocimum	African Journal of biomedical research	International Dec 2024 Vol 27 (ISSN 1119-5096) UGC care list

Annexure II

LIST OF SEMINAR & WORKSHOP ATTENDED

Sr.	Seminar/Workshop	Address	Seminar/workshop
1	International webinar on spatial distributor of covid and its socio ecomonic impact.	Tezpur College in collaboration with UNO, Malawi, Africa held on 8.7.2020	International seminar
2	22nd International Seminar on recent trends of Diversity	ISDR Ranchi, Jharkhand 24-26 Sept. 2022	International seminar
3	National Conference on multidisciplinary approaches	YBN University, Ranchi held on 9-16.Dec.2022	National Seminar
4	National Seminar on TGCST 2024	Magadh Mahila College, & the Indian Science Congress Association patna Chapter held on 20-21 Feb.2024	National Seminar
5	3 days offlineNational workshop on PAGE and DNA Isolation	University Dept. of Zoology TMBU Bhagalpur in collaboration with AERC Govt. of India	National workshop
6	2 days offline workshop on molecular biology & biochemistry technique	IIT Kanpur	Workshop
7	2 days offline workshop on molecular biology & biochemistry technique	IIT Patna	Workshop
8	Research excellence award 2023	Awarded 2023	Research excellence award

PG Department Of Botany

R.D & D.J. College, Munger

(A Constituent Unit of Munger University, Munger 811201)

Ref. DJC/Bot/04/21 Date. 01-09-2021

To Whom It May Concern

This is to certify that equipments and laboratories facilities available in the department of Botany, R.D.&.D.J. College, Munger , will be sufficient to complete the research work of Mr. M.A Jahanger who has applied for registration under my Co- Supervision.

Lav singh
01-09-21

(Dr. Lav Singh)
Head- P.G Deptt. Of Botany
R.D.&.D.J.College, Munger

Head
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Ref. RJC/Bot/06/21 Date. 12-09-2021

To whom it may concern

This is to certify that plants collected by Sri. M.A Jahanger for his Ph.D. research work is the three species of Ocimum (Tulsi). These are identified as *Ocimum tenuiflorum* L. *Ocimum basilicum* L. and *Ocimum gratissimum* L. commonly known as Shyama Tulsi, Sweet basil and Van Tulsi respectively.

We wish for his bright success in future.

(Dr. S. Hassan)
Former Head
Deptt. of Botany
R D & D J College, Munger

Lav Singh
(Dr. Lav Singh)
Head
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